

Response Predictors and Clonal Evolution during Thalidomide Therapy in Myelodysplastic Neoplasms with Increased Blasts

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Abstract

Therapeutic options remain limited for patients with myelodysplastic neoplasms (MDS) with increased blasts who are ineligible for, intolerant of or decline hypomethylating agents (HMAs). We retrospectively investigated the efficacy, predictors of response, survival outcomes and clonal evolution of thalidomide in 56 patients with MDS and increased blasts. Among 50 evaluable patients, the overall response rate was 52.0%, and treatment was well tolerated without grade ≥ 3 non-hematologic adverse events. Multivariable analyses identified male sex, lower baseline lymphocyte count, and higher platelet count as independent predictors of treatment response. Patients who achieved a treatment response experienced significantly longer overall survival (OS) and leukemia-free survival (LFS). Older age, higher IPSS-R category, and *STAG2* mutation independently predicted inferior OS, whereas *SF3B1* mutation was associated with favorable LFS. Serial molecular analyses demonstrated persistent clonal evolution in 58.3% of patients, predominantly through acquisition of new mutations, with *ASXL1* emerging as the most frequently acquired mutation. Overall, thalidomide demonstrated clinical activity with acceptable tolerability in selected patients. Persistent clonal evolution despite morphologic response suggests that the therapeutic benefit of thalidomide reflects disease control rather than eradication of the founding malignant clone, supporting longitudinal molecular monitoring for treatment assessment.

Introduction

Myelodysplastic neoplasms (MDS), formerly referred to as myelodysplastic syndromes, are heterogeneous clonal hematopoietic disorders characterized by cytopenias, bone marrow (BM) dysplasia and an increased risk of evolution to acute myeloid leukemia (AML)⁽¹⁾. Patients with MDS and increased blasts represent a clinically aggressive subgroup characterized by an increased risk of leukemic transformation and inferior survival⁽²⁾. Most are classified as intermediate- or high-risk according to the Revised International Prognostic Scoring System (IPSS-R) or the Molecular IPSS (IPSS-M)^(3–5).

Hypomethylating agents (HMAs), including azacitidine and decitabine, have been widely used in MDS patients with increased blasts, and have become the backbone of disease-modifying therapy^(6, 7). In contrast, real-world outcomes are less favorable than those reported in clinical trials^(8–10). Furthermore, a proportion of patients are unable or unwilling to receive HMA therapy because of frailty, advanced age, comorbidities, concerns regarding treatment tolerance, or personal preference. These unmet clinical needs underscore the importance of developing effective alternative therapeutic strategies.

Thalidomide is an immunomodulatory agent with anti-cytokine, anti-angiogenic and immunoregulatory properties^(11–13). It has primarily been used in lower-risk MDS patients, where it has been shown to improve anemia and reduce transfusion dependence^(14–16). Nevertheless, careful review of previous studies indicates that morphologic responses have also been achieved in a subset of patients with excess blasts, including those classified as refractory anemia with excess blasts (RAEB) and RAEB in

transformation (RAEB-T)^(14–17). These observations suggest that the potential therapeutic activity of thalidomide may extend beyond lower-risk disease. However, data regarding the efficacy of thalidomide in patients with MDS and increased blasts remain scarce.

We retrospectively analyzed 56 MDS patients with increased blasts who received thalidomide treatment at our institution. Clinical responses, survival outcomes and clonal evolution were evaluated to further characterize the role of thalidomide in this patient population.

Methods

Subjects

This retrospective study included consecutive patients with MDS and increased bone marrow blasts who received thalidomide at our center between August 2016 and May 2026. According to the 2022 WHO classification⁽¹⁸⁾, patients were classified as MDS with increased blasts-1 (MDS-IB1), MDS with increased blasts-2 (MDS-IB2), MDS with biallelic *TP53* inactivation (MDS-bi*TP53*), or MDS with fibrosis (MDS-f). The diagnosis of MDS was established based on the minimal diagnostic criteria⁽¹⁹⁾. Conventional cytogenetic analysis was available for 52 patients (92.9%) at diagnosis, and risk stratification was performed using the Revised International Prognostic Scoring System (IPSS-R)⁽³⁾. Molecular risk assessment according to the Molecular International Prognostic Scoring System (IPSS-M) was conducted in patients with available sequencing data⁽⁴⁾. According to the IPSS-M, patients in the moderate-high, high, and very-high risk categories were classified as higher-risk MDS (HR-MDS)⁽⁴⁾. Follow-up information was available for 52 patients (92.9%).

Response assessment

Treatment response evaluation was performed according to the 2023 International Working Group (IWG) criteria for MDS⁽²⁰⁾. Only patients who received thalidomide treatment for at least 3 months were included in the efficacy analysis, and the best response achieved during thalidomide therapy was used for efficacy analyses. Patients without evaluable response assessments due to loss to follow-up or the absence of post-treatment bone marrow evaluation were excluded from response analyses. Because this was a retrospective study, bone marrow evaluations were performed according to routine clinical practice rather than at predefined protocol-specified time points.

The primary endpoint was overall response rate (ORR), which included complete remission (CR), CR equivalent, CR with limited count recovery (CR_L), including CR_{bi} (CR bilineage) and CR_{uni} (CR unilineage), CR with partial hematologic recovery (CRh), partial remission (PR), and hematologic improvement (HI). Patients achieving any of these responses were categorized as responders; all others were classified as non-responders. Transfusion independence was evaluated based on the IWG 2023 criteria⁽²⁰⁾.

Targeted gene sequencing

Targeted next-generation sequencing (NGS) was performed using genomic DNA extracted from bone marrow mononuclear cells. Sequencing libraries were analyzed on the MiSeq platform (Illumina, San Diego, CA, USA), as previously described^(21, 22).

Variants were annotated and filtered against public databases, including 1000 Genomes, ESP6500, COSMIC, PolyPhen and SIFT. The overall sequencing coverage exceeded 99%, with a mean depth of 800×. Variants with a variant allele frequency (VAF) $\geq 2\%$ were considered positive. *TP53* allelic state was determined as described⁽¹⁸⁾. Details of the targeted gene panel are provided in **Table S1**.

Statistics

Continuous variables were compared using the Mann–Whitney U test, whereas categorical variables were analyzed using the χ^2 test or Fisher's exact test. Variables associated with treatment response were evaluated using univariable logistic regression, and those with $P < 0.10$ were entered into a multivariable logistic regression model using a forward stepwise likelihood-ratio method. Overall survival (OS) and leukemia-free survival (LFS) were estimated using the Kaplan-Meier method and compared using the log-rank test. Univariable and multivariable Cox proportional hazards regression models were performed to identify prognostic factors for OS and LFS. Variables with $P < 0.10$ in univariable Cox analyses were entered into multivariable Cox regression models. All statistical analyses were performed using SPSS version 27.0, GraphPad Prism 8.0, and RStudio. Two-sided P values < 0.05 were considered statistically significant.

Results

1. Patient characteristics

A total of 56 MDS patients with increased blasts who received thalidomide treatment were included in this study. The median age at diagnosis was 67 years (range, 36–83 years), and 39 patients (69.6%) were male. MDS-IB1 was the most common subtype (58.9%), followed by MDS-IB2 (21.4%), MDS-f (12.5%) and MDS-bi*TP53* (7.1%). According to the IPSS-M, 33 patients (58.9%) were classified as higher-risk MDS. Detailed baseline demographic, clinical, laboratory and cytogenetic characteristics are presented in Table 1.

Table 1
Demographic and Hematologic Characteristics of MDS patients treated with thalidomide according to treatment response

Characteristic	Total (n = 56)	Non-responder group (n = 24)	Responder group (n = 26)	P-value
Age (years) *	67 (36–83)	67 (45–75)	66 (36–83)	0.930
Sex n (%)				0.019
Male	39 (69.6)	13 (54.2)	22 (84.6)	
Female	17 (30.4)	11 (45.8)	22 (15.4)	
WHO 2022 subtypes				0.410
MDS-IB1	33 (58.9)	14 (58.3)	19 (73.1)	
MDS-IB2	12 (21.4)	5 (20.8)	5 (19.2)	
MDS-f	7 (12.5)	3 (12.5)	2 (7.7)	
MDS-bi <i>TP53</i>	4 (7.1)	2 (8.3)	0 (0)	
IPSS-R karyotype				0.165
Very good	1 (1.8)	0 (0)	1 (4.2)	
Good	34 (60.7)	13 (59.1)	19 (79.2)	
Intermediate	5 (8.9)	3 (13.6)	2 (8.3)	
Poor	4 (7.1)	2 (9.1)	2 (8.3)	
Very poor	8 (14.3)	4 (18.2)	0 (0)	
Complex karyotype	9 (16.1)	5 (22.7)	0 (0)	0.019
IPSS-R Prognostic Risk Score				0.320
Very low	0 (0)	0 (0)	0 (0)	
Low	5 (8.9)	1 (4.8)	4 (16.7)	
Intermediate	11 (19.6)	6 (28.6)	5 (20.8)	
High	21 (37.5)	8 (38.1)	12 (50)	
Very high	13 (23.2)	6 (28.6)	3 (12.5)	
IPSS-M Prognostic Risk Score				0.255
Very low	1 (1.8)	0 (0)	1 (4.2)	
Low	8 (14.3)	2 (9.5)	6 (25)	

Characteristic	Total (n = 56)	Non-responder group (n = 24)	Responder group (n = 26)	P-value
Moderate low	8 (14.3)	3 (14.3)	5 (20.8)	
Moderate high	6 (10.7)	2 (9.5)	4 (16.7)	
High	14 (25)	10 (47.6)	4 (16.7)	
Very high	13 (23.3)	4 (19.0)	4 (16.7)	
HR-MDS	33 (58.9)	16 (76.2)	12 (50.0)	0.071
BM fibrosis grade, n (%)				0.136
MF-0	24 (48.0)	8 (33.3)	16 (61.5)	
MF-1	21 (42.0)	13 (54.2)	8 (30.8)	
MF-2/3	5 (10.0)	3 (12.5)	2 (7.6)	
Auer rods	4 (7.1)	3 (12.5)	0 (0)	0.110
Hemoglobin (g/L) *	83.5 (42–141)	91 (57–139)	85 (46–141)	0.839
MCV (fL) *	101.9 (71–128)	98.1 (71–123.8)	103 (94.4–128)	0.048
RDW-CV (%) *	16 (11.8–30.3)	16.85 (13.3–30.3)	15.2 (11.8–20.9)	0.066
WBC ($\times 10^9/L$) *	2.6 (1–10.0)	3.5 (1.0–10.0)	2.2 (1.1–6.7)	0.031
ANC ($\times 10^9/L$) *	0.9 (0.1–8.6)	1.5 (0.1–8.6)	0.9 (0.2–5.6)	0.032
LYM ($\times 10^9/L$) *	1.1 (0.4–2.6)	1.5 (0.5–2.8)	1.0 (0.4–2.6)	0.016
Platelets ($\times 10^9/L$) *	55 (6–364)	44 (6–260)	66 (31–364)	0.055
BM blasts (%) *	6.6 (3.2–16.5)	6.1 (3.2–12)	6.6 (5–15)	0.385
LDH (U/L) *	299 (144–1287)	325 (160–668)	252 (144–1287)	0.878
EPO (mIU/ml) *	422 (5–5164)	273 (5–4581)	705 (11–5164)	0.435
Ferritin (ng/ml) *	715 (118–3346)	758 (118–3346)	648 (148–1955)	0.630

Abbreviations: MDS, myelodysplastic syndrome; BMF: bone marrow fibrosis; WHO: World Health Organization; IB: increased blasts; IPSS-R: International Prognostic Scoring System-Revised; HR-MDS:

higher-risk MDS, defined as IPSS-M moderate-high, high, and very high risk categories; MCV, mean corpuscular volume; RDW-CV, red cell distribution width-coefficient of variation; WBC, white blood cell; ANC, absolute neutrophil count; LYM, lymphocyte; BM, bone marrow; LDH, lactate dehydrogenase; EPO, erythropoietin;

P value: Non-responder group vs. Responder group; Responders were defined as patients achieving complete remission (CR), CR equivalent, CR with limited count recovery, CR with partial hematologic recovery (CRh), partial remission (PR), or hematologic improvement (HI) according to the IWG 2023 criteria; all remaining patients were classified as non-responders.

*: Median (range)

The mutational landscape of the cohort is shown in Fig. 1. The most frequently mutated genes were *ASXL1* (21%), *DDX41* (18%), *TET2* (11%), *STAG2* (11%), *U2AF1* (11%) and *RUNX1* (11%) (Fig. 1). No detectable mutations were identified in 8 patients (14.3%).

2. Treatment response and predictors of response

2.1 Treatment exposure and response

The median duration of thalidomide treatment was 11.5 months (range, 3-100 months), and the daily dose ranged from 50 to 100 mg. Among the 56 patients, 50 were evaluable for response assessment, whereas 6 patients were excluded from response analyses because of loss to follow-up or the absence of post-treatment bone marrow evaluation.

According to the IWG 2023 response criteria, the overall response rate (ORR) was 52.0% (26/50), including 8 patients who achieved CR (16.0%), 8 who achieved CRh (16.0%) and 10 who achieved CR_L (20.0%). Among the CR_L responses, 8 were classified as CR_{uni} and 2 as CR_{bi}. No patients achieved PR or isolated HI. Nine patients (18.0%) were classified as no response, and disease progression was observed in 15 patients (30.0%).

Thalidomide was generally well tolerated. The most common adverse events were dizziness (38/56, 67.9%), constipation (29/56, 51.8%), and peripheral edema (15/56, 26.8%), all of which were grade 1–2 and manageable with supportive care or dose adjustment. No grade ≥ 3 non-hematologic adverse events, treatment-related deaths, or unexpected toxicities were observed. During follow-up, 17 patients (30.4%) subsequently received hypomethylating agents or chemotherapy, and 4 patients (7.1%) underwent allogeneic hematopoietic stem cell transplantation.

2.2 Comparison between responders and non-responders

To identify factors associated with treatment response, baseline characteristics were compared between responders and non-responders (Table 1). Responders were more frequently male than non-responders (84.6% vs. 54.2%, *P* = 0.019). Compared with non-responders, responders had a higher mean

corpuscular volume (MCV; $P = 0.048$), lower white blood cell counts ($P = 0.031$), lower absolute neutrophil counts ($P = 0.032$) and lower lymphocyte counts ($P = 0.016$). Platelet counts tended to be higher ($P = 0.055$), whereas red cell distribution width–coefficient of variation (RDW-CV) tended to be lower ($P = 0.066$) in responders.

Complex karyotypes were identified exclusively in the non-responder group ($P = 0.019$). A trend toward inferior response was observed among patients with higher-risk IPSS-M categories (76.2% vs. 50.0%, $P = 0.071$), although this association did not reach statistical significance. No significant differences were observed in age, WHO 2022 subtype, IPSS-R risk group, fibrosis, hemoglobin level, LDH, EPO, or ferritin levels.

The mutational profiles of responders and non-responders were subsequently compared (Fig. 2). It seems *ASXL1* mutations were more frequently observed among responders (35% vs. 12%, $P = 0.067$), whereas *STAG2* (4% vs. 21%, $P = 0.093$) and *SRSF2* mutations (0% vs. 12%, $P = 0.063$) tended to be enriched in non-responders. No statistically significant differences were observed for other recurrently mutated genes.

2.3 Analysis of factors associated with response

Variables associated with treatment response in univariable analyses ($P < 0.10$) were entered into a forward stepwise logistic regression model using the likelihood-ratio method (Table 2). Lower baseline lymphocyte count (OR, 0.132; 95% CI, 0.032–0.555; $P = 0.006$) and higher platelet count (OR, 1.014; 95% CI, 1.002–1.027; $P = 0.025$) remained independently associated with treatment response. Female sex was independently associated with a lower likelihood of achieving a response (OR, 0.111; 95% CI, 0.017–0.706; $P = 0.020$), indicating that male patients were more likely to respond to thalidomide treatment.

Table 2
Clinical and molecular factors associated with treatment response

Variable	Univariable		Multivariable	
	OR (95% CI)	P	OR (95% CI)	P
Male sex	0.215 (0.057–0.816)	0.019	0.111 (0.017–0.706)	0.020
Complex karyotype	NE*	0.019		
Higher-risk IPSS-M	0.313 (0.087–1.128)	0.071		
MCV	1.060 (0.991–1.135)	0.048		
RDW-CV	0.802 (0.634–1.015)	0.066		
WBC	0.886 (0.739–1.061)	0.031		
ANC	0.861 (0.661–1.121)	0.032		
LYM	0.355 (0.129–0.980)	0.016	0.132 (0.032–0.555)	0.006
Platelet	1.010 (0.999–1.021)	0.055	1.014 (1.002–1.027)	0.025
<i>ASXL1</i>	3.706 (0.865–15.873)	0.067		
<i>SRSF2</i>	NE*	0.063		
<i>STAG2</i>	0.152 (0.016–1.411)	0.093		
Abbreviations: OR: odds ratio. CI: confidence interval;				
NE: not estimable because no Responders carried a complex karyotype or an <i>SRSF2</i> mutation.				

3. Prognostic factors for survival

After a median follow-up of 36 months (95% CI, 27–45 months), 25 patients (44.6%) had died and 11 (19.6%) had progressed to AML. The median OS for the entire cohort was 47.0 months (95% CI, 32.1–61.9 months; Fig. 3A). The estimated 2-year OS rate was 67.4%. The median LFS for the entire cohort was 30.0 months (95% CI, 21.4–38.6 months; Fig. 3B).

Kaplan-Meier analyses demonstrated significantly superior survival outcomes among patients who achieved a thalidomide response. Responders had significantly longer OS than non-responders (log-rank $P = 0.03$; Fig. 3C). Likewise, responders exhibited significantly prolonged LFS compared with non-responders (log-rank $P < 0.001$; Fig. 3D).

Univariable Cox regression analyses identified variables associated with OS, including age, MCV, IPSS-R risk category, treatment response, and *TP53*, *IDH2*, *RUNX1*, *STAG2*, and *SETBP1* mutations (all P values < 0.10 ; Table 3). These variables with $P < 0.10$ in univariable analyses were subsequently entered into the multivariable Cox regression.

Table 3

Univariable and Multivariable Analysis of Prognostic Factors for Overall Survival in Patients With MDS

Variable	Univariable		Multivariable	
	HR (95% CI)	P	HR (95% CI)	P
Age	1.040 (0.996–1.085)	0.054	1.088 (1.013–1.169)	0.021
MCV	0.964 (0.925–1.005)	0.091		
LDH	1.003 (1.001–1.005)	0.017		
Ferritin	1.001 (1.000–1.001)	0.057		
IPSS-R risk category	1.520 (0.929–2.486)	0.083	2.282 (1.137–4.581)	0.020
Auer rods	0.169 (0.018–1.565)	0.075		
Thalidomide response status	2.569 (1.058–6.238)	0.030		
<i>TP53</i>	1.00 (0.002–639.28)	< 0.0001		
<i>IDH2</i>	5.299 (0.662–42.42)	0.076		
<i>STAG2</i>	4.485 (1.167–17.24)	0.016	32.99 (1.55–704.46)	0.025
<i>RUNX1</i>	2.684 (0.878–8.206)	0.069		
<i>SETBP1</i>	4.440 (0.946–20.835)	0.037		
Abbreviations: HR, hazard ratio. CI: confidence interval.				

In multivariable analysis, older age (HR, 1.088; 95% CI, 1.013–1.169; $P = 0.021$), higher IPSS-R risk category (HR, 2.282; 95% CI, 1.137–4.581; $P = 0.020$), and *STAG2* mutation (HR, 32.99; 95% CI, 1.55–704.46; $P = 0.025$) remained independently associated with inferior OS (Table 3).

For LFS, univariable Cox regression analyses showed that older age, lower MCV, higher LDH levels, the presence of Auer rods, failure to achieve a thalidomide response, a higher number of mutations, *TP53*, *SRSF2* and *STAG2* mutations were associated with inferior LFS, whereas *SF3B1* mutation was associated with favourable LFS (**Table S2**). Variables with $P < 0.10$ in univariable analyses were subsequently entered into a forward stepwise Cox proportional hazards model using the likelihood-ratio method. *SF3B1* mutation remained the only independent prognostic factor associated with improved LFS (HR, 0.033; 95% CI, 0.003–0.368; $P = 0.005$) (**Table S2**).

4. Clonal evolution during thalidomide treatment

Serial molecular analyses were available for 12 patients (**Table S3**). Diverse patterns of clonal evolution were observed during thalidomide treatment. Four patients (33.3%) acquired new mutations, two (16.7%) exhibited concurrent mutation loss and acquisition, one (8.3%) developed multiple newly acquired mutations, one (8.3%) showed clonal expansion with increased variant allele frequencies, two (16.7%) maintained stable clonal profiles, and one (8.3%) demonstrated mutation loss only. Overall, acquisition of at least one new mutation occurred in eight patients (66.7%). *ASXL1* was the most frequently acquired mutation, whereas most newly acquired mutations involved genes associated with RNA splicing or epigenetic regulation.

Clonal evolution was observed in both responders and non-responders. Two patients subsequently progressed to AML, both of whom acquired additional mutations during follow-up, whereas no AML transformation occurred among patients with stable clonal profiles. Representative examples of these evolutionary patterns are illustrated in Fig. 4.

Discussion

MDS patients with increased blasts represent a challenging population characterized by a high risk of leukemic transformation and generally have poor survival^(1, 2). Although HMAs currently remain the standard non-transplant treatment^(6, 7), a substantial proportion of patients are ineligible for, intolerant of, or unwilling to receive HMAs. Moreover, recent studies have suggested that despite the introduction of HMAs, overall survival of patients with MDS has not substantially improved in real-world practice, particularly among patients with higher-risk disease⁽¹⁰⁾. Given that treatment-related toxicity and limited treatment tolerance remain major challenges in this predominantly older and medically frail population, there is a continuing need for effective therapies with a more favorable tolerability profile. Consequently, identifying effective alternative therapeutic strategies for these patients remains clinically relevant.

Immune dysregulation is increasingly recognized as a hallmark of higher-risk MDS, characterized by impaired cytotoxic T-cell function, expansion of immunosuppressive cell populations, and defective anti-leukemic immune surveillance^(23–26). As an immunomodulatory agent with anti-inflammatory and anti-angiogenic properties, thalidomide may partially restore immune homeostasis within the bone marrow^(11–13). Although its activity has been more extensively studied in lower-risk disease, several reports have suggested potential clinical benefit in MDS patients with increased blasts^(14–17). However, these studies included relatively small and heterogeneous populations and lacked comprehensive molecular characterization. Therefore, we evaluated the efficacy of thalidomide in a cohort of MDS patients with increased blasts and further explored clinical and molecular factors associated with treatment outcomes, as well as clonal evolution during therapy.

Consistent with previous reports suggesting potential activity of thalidomide in patients with increased blasts^(14–16), the present study further supports its clinical efficacy by demonstrating an overall response rate of 52.0% with acceptable tolerability. To further identify patients most likely to benefit from thalidomide, we investigated baseline clinical and molecular predictors of treatment response. that Male

sex, lower baseline lymphocyte count, and higher platelet count were found independently associated with treatment response.

Previous studies have similarly reported that responders to thalidomide tended to have higher pretreatment platelet counts and lower marrow blast percentages than non-responders⁽¹⁴⁾, suggesting that patients with better preserved hematopoietic reserve and a lower disease burden are more likely to benefit from thalidomide therapy.

Besides, lower baseline lymphocyte counts independently predicted treatment response in our cohort. This observation raises the possibility that peripheral lymphocyte count may serve as a simple surrogate marker of the immune microenvironment. Accumulating evidence indicates that immune dysregulation is a fundamental feature of MDS pathogenesis, characterized by aberrant T-cell activation, excessive inflammatory cytokine production, and disruption of the bone marrow immune microenvironment^(11, 12, 27–29). Although only absolute lymphocyte counts were available, these findings suggest that future studies incorporating CD4⁺ and CD8⁺ T-cell subsets, regulatory T cells, and cytokine profiling may help identify patients most likely to benefit from thalidomide.

Male sex was also independently associated with treatment response. Although the biological basis for this finding remains uncertain, accumulating evidence suggests that sex-related differences in immune regulation and inflammatory responses may influence therapeutic efficacy in hematologic malignancies. However, this observation requires validation in larger prospective cohorts. Collectively, these findings suggest that the therapeutic efficacy of thalidomide is influenced by both disease biology and host immune characteristics, with patients exhibiting less aggressive disease features appearing more likely to benefit from treatment.

We found that patients who achieved a treatment response experienced significantly longer OS and LFS than non-responders, indicating that thalidomide-induced response translates into durable clinical benefit. Although treatment response was not retained as an independent predictor of survival after adjustment for baseline characteristics, this finding is not unexpected because response is likely influenced by underlying disease biology. Nevertheless, these results highlight the clinical importance of achieving an effective response in MDS patients with increased blasts treated with thalidomide.

Consistent with previous studies, older age and higher IPSS-R category remained independently associated with inferior OS, highlighting that both patient-related and disease-related characteristics contribute to long-term outcomes despite thalidomide treatment^(4, 30–32). Beyond these clinical prognostic factors, *STAG2* mutation emerged as an independent adverse prognostic predictor for OS. *STAG2* is a recurrent mutated cohesin-complex gene that was significantly associated with adverse survival and an increased risk of leukemic progression^(4, 31, 32). Our findings demonstrate that the adverse prognostic impact of *STAG2* persists even among patients treated with thalidomide, suggesting that unfavorable molecular features continue to influence long-term outcomes despite therapy.

SF3B1 mutation was the only independent factor associated with favorable LFS in our cohort. This finding is consistent with previous studies demonstrating that *SF3B1*-mutated MDS generally exhibits a lower risk of leukemic transformation and superior outcomes^(4, 33). Collectively, these findings emphasize that molecular features continue to shape long-term outcomes even after treatment has been initiated, underscoring the importance of integrating genomic profiling into therapeutic decision-making and risk stratification.

One strength of the study is the availability of serial molecular analyses, which allowed dynamic assessment of clonal evolution during thalidomide treatment. More than half of the patients exhibited ongoing clonal evolution despite therapy.

Consistent with previous studies, acquisition of new mutations was the predominant pattern of clonal evolution^(32, 33). Newly acquired mutations in our cohort were enriched in genes involved in chromatin regulation and RNA splicing, including *ASXL1*, *SRSF2*, *SF3B1*, and *PHF6*, supporting previous genomic studies identifying these pathways as major drivers of MDS evolution⁽³²⁾. Given that only two patients progressed to AML, the observed mutational profile likely reflects continued evolution within high-risk MDS rather than terminal leukemic transformation. Although limited by sample size, these findings support serial molecular monitoring as a valuable complement to morphologic response assessment.

This study has several limitations. First, its retrospective single-center design introduces the possibility of selection bias. Second, serial molecular analyses were available only in a subset of patients, limiting definitive conclusions regarding clonal evolution. Third, immune profiling was not performed, precluding mechanistic investigation of the observed association between lymphocyte count and treatment response. Finally, the relatively small sample size limits statistical power, particularly for mutation-specific analyses. Prospective multi-center studies incorporating comprehensive immunologic and genomic profiling are warranted.

Taken together, our findings suggest that thalidomide is a feasible therapeutic option for selected patients with MDS and increased blasts who are ineligible for, intolerant of, or unwilling to receive HMA therapy because of its favorable tolerability profile. Baseline clinical and molecular characteristics may help identify patients most likely to benefit from treatment. However, persistent clonal evolution despite clinical responses indicates that thalidomide primarily provides disease control rather than eradication of the founding malignant clone, supporting serial molecular monitoring to complement conventional response assessment and improve longitudinal disease evaluation.

Declarations

Funding information

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Conflict of interests

All authors declare no conflict of interests.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly due to privacy or ethical restrictions.

Ethics approval statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Shanghai Sixth People's Hospital (protocol code 2021-033, date of approval 25 February 2021). All informed consent was obtained from the subjects.

Author Contributions

C.K.C. conceived and designed the study. J.Y.W., Y.S.Z., X.C.Z. and J.G. collected and analyzed the data. R.J.W., L.S., S.D.Z., Z.Z., L.X.S., Q.H. and L.Y.W. recruited patients and collected clinical data. J.Y.W. drafted the manuscript. D.W. and C.K.C. critically revised the manuscript. All authors reviewed and approved the final version.

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Figures

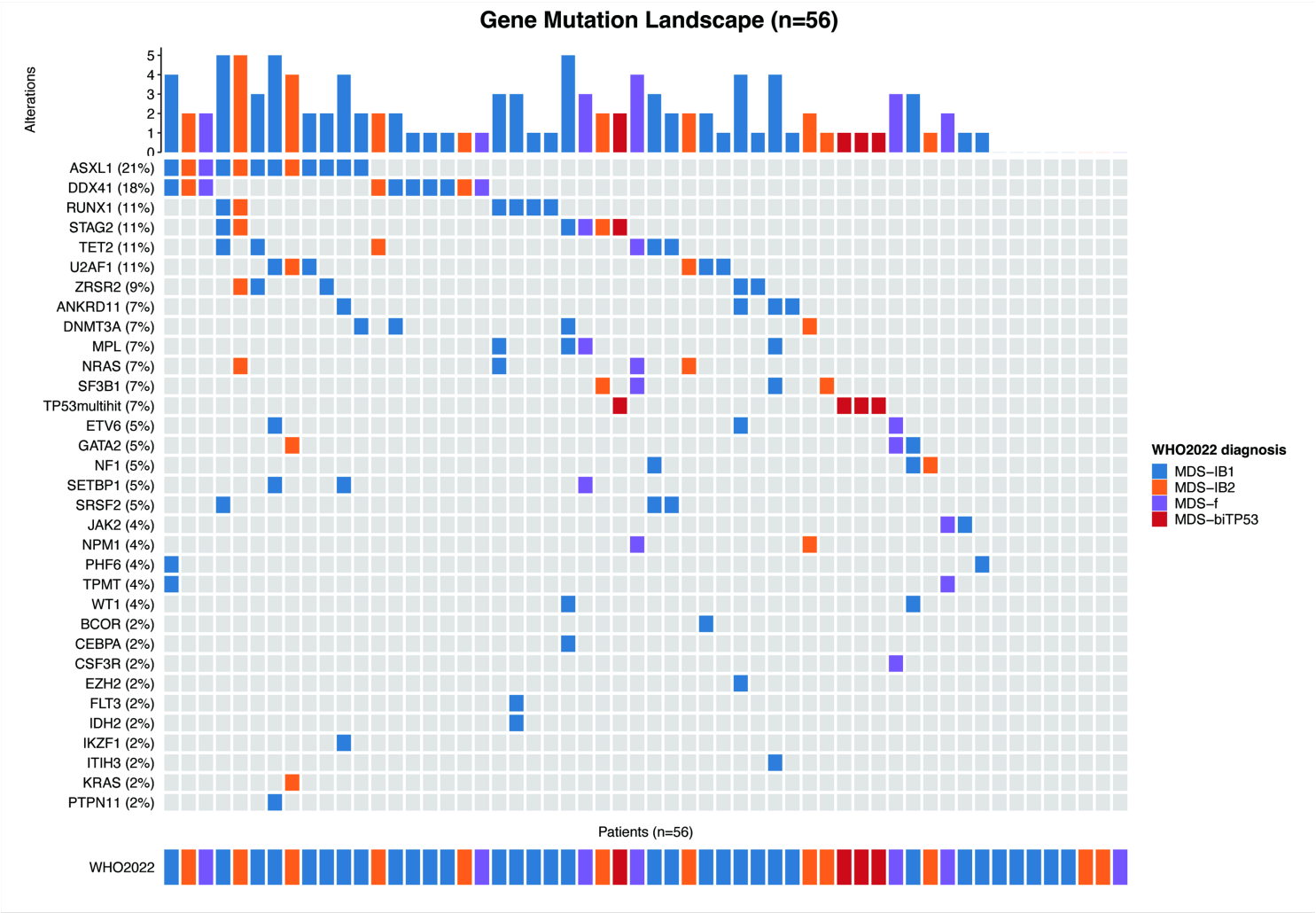


Figure 1

Baseline mutational landscape of patients with MDS and increased blasts. Heat map showing the baseline mutational profiles of the 56 patients included in this study. Each column represents an individual patient, and each row represents a recurrently mutated gene. The upper bar plot indicates the number of mutations per patient, and the bottom annotation indicates the WHO 2022 disease subtype. Abbreviations: MDS with increased blasts 1 (MDS-IB1), MDS with increased blasts 2 (MDS-IB2), MDS with fibrosis (MDS-f), and MDS with biallelic *TP53* inactivation (MDS-bi*TP53*).

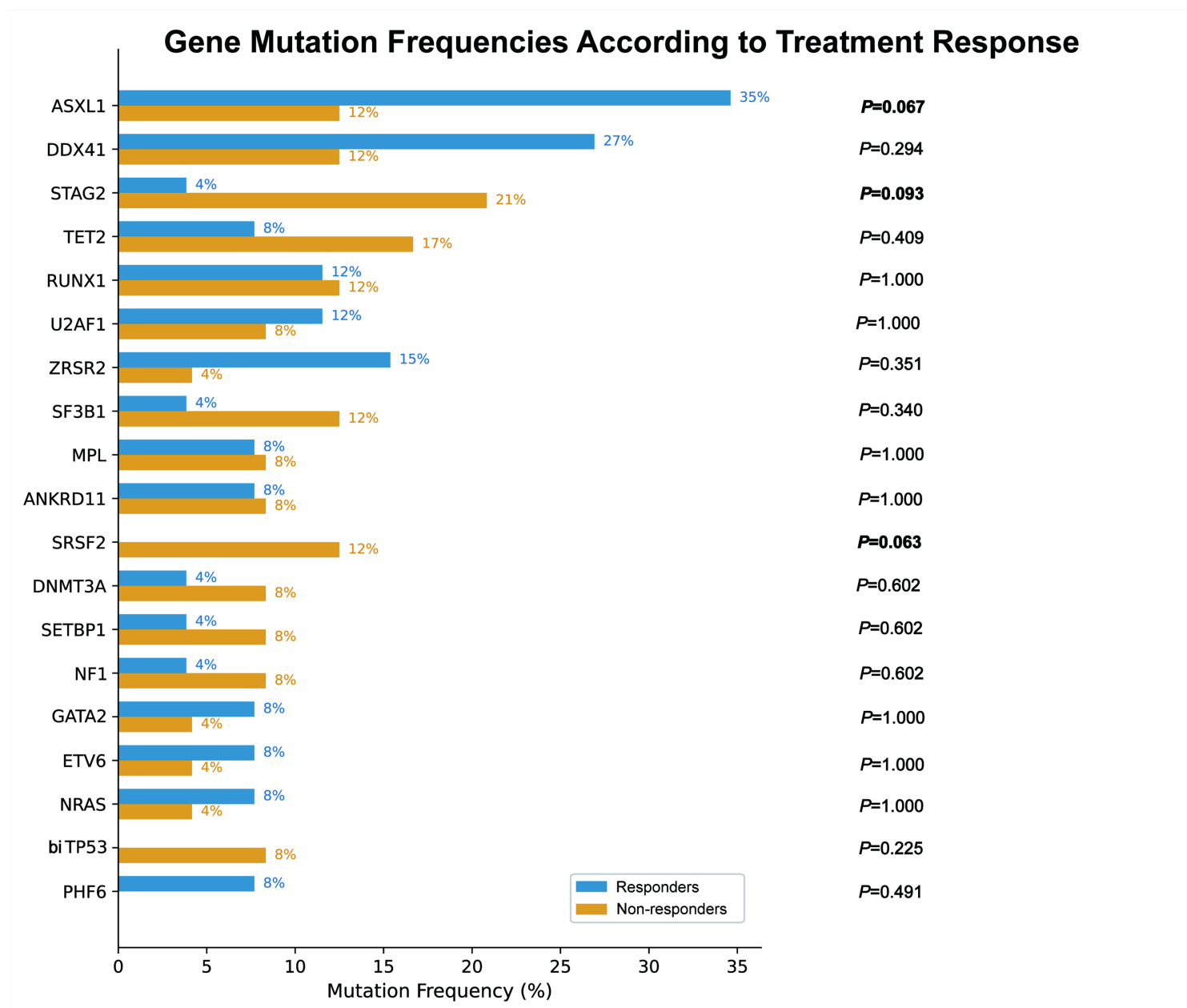


Figure 2

Baseline mutation frequencies according to treatment response. Mutation frequencies of recurrently mutated genes in responders and non-responders at baseline. Only genes mutated in at least two patients are shown. *P* values were calculated using Fisher's exact test.

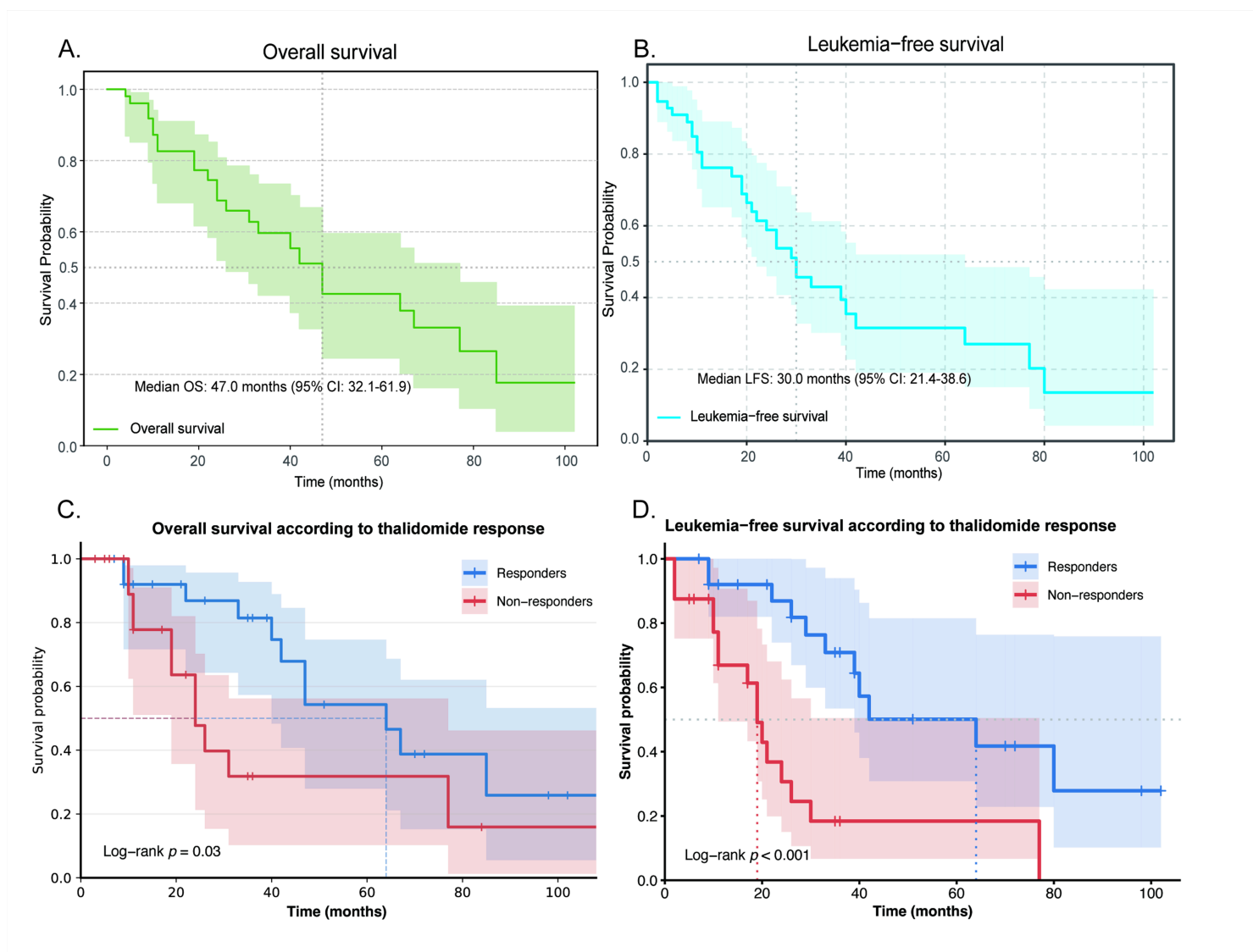


Figure 3

Survival outcomes of patients with MDS and increased blasts treated with thalidomide. Kaplan-Meier analyses of overall survival (OS) (A) and leukemia-free survival (LFS) (B) for the entire cohort. Kaplan-Meier curves comparing OS (C) and LFS (D) according to treatment response. Shaded areas represent 95% confidence intervals. P values were calculated using the log-rank test.

Representative patterns of clonal evolution during thalidomide treatment

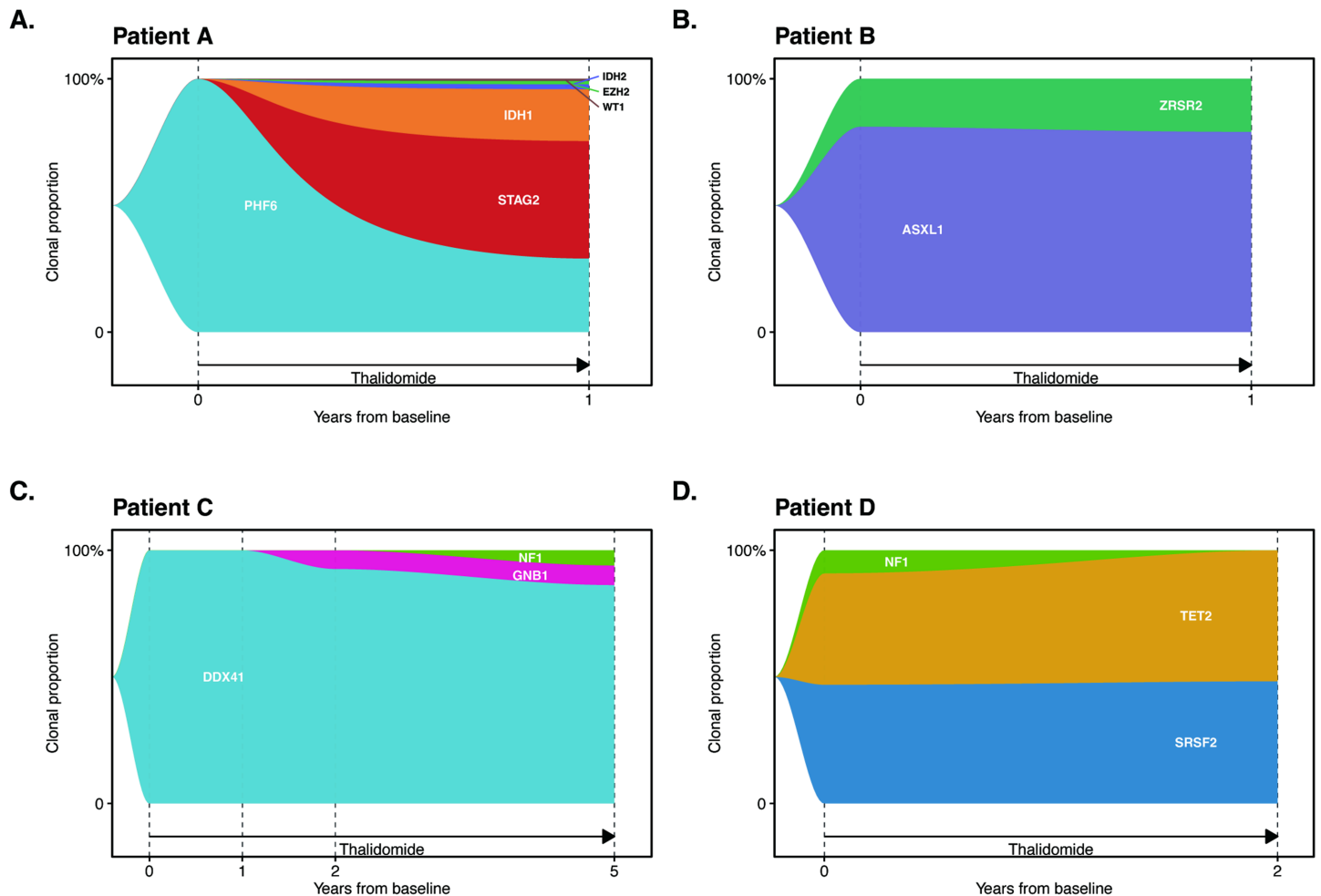


Figure 4

Representative patterns of clonal evolution during thalidomide treatment. Fish plots illustrating four representative patterns of clonal evolution identified by paired molecular analyses. The width of each colored area represents the relative proportion of individual clones over time. Patient A represents multiple newly acquired mutations, Patient B demonstrates a stable clonal architecture, Patient C illustrates acquisition of new mutations, and Patient D demonstrates loss of pre-existing mutations. The duration of thalidomide treatment is indicated below each plot.

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