Current use of androgens in bone marrow failure disorders: a report from the Severe Aplastic Anemia Working Party of the European Society for Blood and Marrow Transplantation

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Abstract

Androgens represent the historical therapeutic backbone of bone marrow failure (BMF) syndromes. However, their role has rarely been analyzed in a prospective setting, and systematic and long-term data regarding their usage, effectiveness and toxicity in both acquired and inherited BMF are currently unavailable. Here, taking advantage of a unique disease-specific international dataset, we retrospectively analyzed the largest cohort so far of BMF patients who received androgens before or in the absence of an allogeneic hematopoietic cell transplantation (HCT), re-evaluating their current use in these disorders. We identified 274 patients across 82 European Society for Blood and Marrow Transplantation (EBMT) affiliated centers: 193 with acquired (median age 32 years) and 81 with inherited (median age 8 years) BMF. With a median duration of androgen treatment of 5.6 and 20 months, respectively, complete and partial remission rates at 3 months were 6% and 29% in acquired and 8% and 29% in inherited disorders. Five-year overall survival and failure-free survival (FFS) were respectively 63% and 23% in acquired and 78% and 14% in inherited BMF. Androgen initiation after second-line treatments for acquired BMF, and after >12 months post diagnosis for inherited BMF were identified as factors associated with improved FFS in multivariable analysis. Androgen use was associated with a manageable incidence of organ-specific toxicity, and low rates of solid and hematologic malignancies. Sub-analysis of transplant-related outcomes after exposure to these compounds showed probabilities of survival and complications similar to other transplanted BMF cohorts. This study delivers a unique opportunity to track androgen use in BMF syndromes and represents the basis for general recommendations on this category of therapeutics on behalf of the Severe Aplastic Anemia Working Party of the EBMT.

Introduction

Anabolic steroids have been in use for several decades as a class of therapeutics in both inherited and acquired bone marrow failure (BMF), due to their pleiotropic effects on erythropoiesis, telomere regulation, and immune homeostasis.[1] The potential role of androgens in this condition was initially based on the observation, back at the beginning of the 20th century, of some sporadic cases of spontaneous remission in young boys with aplastic anemia (AA) at the onset of puberty.[2,3] These spontaneous hematologic recoveries, together with some reports describing the occurrence of myeloid hyperplasia in breast cancer patients receiving testosterone,[4] set the stage for the use of androgen-based protocols alone or in association with various immunosuppressive regimens for the treatment of acquired or inherited BMF,[5-8] with data generated from randomized trials which have been conducted since the early 1980s.[9,10] Specifically, the discordant results produced by these historical trials, showing in one case no benefits in adding androgens to anti-thymocyte globulin (ATG)[9] and in another case the superiority of the association in response but not in overall survival,[10] did not promote the use of androgens as front-line treatment in an idiopathic setting.

Synthetic and natural anabolic steroids act via the interaction with androgen receptors (AR), members of the steroid hormone nuclear receptor family and ligand-dependent nuclear transcription factors.[11] These structures are widely expressed in many tissues ensuring a large range of biological effects, including maintenance of the musculoskeletal, cardiovascular, reproductive, neural, hematopoietic, and immune systems.[1,11-13]

Effects on hematopoiesis in the context of BMF may depend on pleiotropic mechanisms, including: 1) stimulation of erythropoietin (EPO) receptor expression on erythroid progenitors and increased iron mobilization via hepcidin inhibition;[14-16] 2) telomere elongation in hematopoietic stem cells (HSC) via binding to the estrogen response elements (ERE) present in the *TERT* gene promoter after estrogen aromatization, indirectly impacting cell survival and proliferation signaling;[6,8,17-20] 3) modulation of immune differentiation programs, reducing T-cell activation, Ig production, proinflammatory cytokines, and thymic cellularity.[21-23] It is thus not unlikely that the effect on each BMF type is mediated by the activation of different pathways. Furthermore, despite the observation of hematologic improvement under different androgenic analogs, the mechanism of specific molecules remains poorly understood. For instance, danazol has been reported to be able to induce telomere elongation,[6,8,17] despite the fact it is structurally not an aromatizable steroid, and is thus unable to interact directly via ERE with the *TERT* gene promoter.[24,25] The biological consequences of AR stimulation on hematopoiesis have mostly been investigated in some inherited disorders including dyskeratosis congenita (DC) and Fanconi anemia (FA), where androgens still represent one of the main non-transplant therapeutical backbones, achieving satisfactory outcomes.[7,17,26,27] In a setting of acquired disorders, they were used prior to the availability of ATG and later on, in combination with it as front-line therapy or as part of subsequent therapeutic approaches.[5,9,10,28-33]

Danazol, 3-alpha-etiocholanolone, nandrolone, oxymetholone, oxandrolone are some of the anabolic androgenic compounds used in BMF, showing variable response and toxicity profiles.[6,8,19,28-30,32,34] Although integrated into the therapeutic armamentarium for acquired and inherited BMF, many outstanding questions remain unanswered concerning the ideal molecule, optimal dose, timing, disease subgroup, place in the treatment algorithm, and factors influencing response. Moreover, the lack of a systematic analysis of long-term outcomes of patients receiving androgens precludes any clear conclusions as to their safety profile, especially in terms of oncogenic potential and toxicity.

Here, we assembled the largest international cohort of patients receiving androgens for acquired or inherited BMF on behalf of the Severe Aplastic Anemia Working Party (SAAWP) of the European Society for Blood and Marrow Transplantation (EBMT) in order to track their current real-life use, indications, efficacy, toxicity, and long-term effects.

Methods

Study design and aims

This is a retrospective multicenter study based on the collection of clinico-biological data of patients receiving androgens for a BMF disorder in EBMT-affiliated centers. This research was conducted under the Institutional Review Board (IRB) and local ethics committees of all the centers involved, and all patients included agreed to participate in clinical and biological research studies conducted on behalf of the EBMT. All the procedures were carried out under the legacy and the ethical principles of the Declaration of Helsinki. Patients were included based on data provided by centers concerning the use of anabolic steroids from 1997 to 2021 as any line before or in the absence of hematopoietic cell transplantation (HCT). The primary objective was to assess the response to androgens in patients diagnosed with acquired and inherited BMF. Secondary aims were to describe the background use of androgens, to assess clinical outcomes in patients receiving androgens (including in a transplant setting), to determine prognostic factors associated with failure, and to evaluate early and late toxicities.

Data collection

Eligible patients were identified through the SAAWP database, a disease-specific database based on collection of clinical and biological data of patients diagnosed with acquired and inherited BMF in EBMT-affiliated centers. Non-European participating centers included hospitals in Pakistan, Russia, China, Saudi Arabia, and Turkey.

An electronic clinical report form (eCRF) was sent to all participating centers to collect information on demographics, comorbidities, primary diagnosis and classification of BMF, baseline blood product transfusions, number and type of previous treatments, date of response, timing of androgen start, type and posology of androgen treatment, response at 3 and 6 months after androgen treatment, time and duration of the response, time to next treatment, reason for stopping, time to transplant, androgen-related early and late toxicity, clonal evolution, and secondary neoplastic events, as well as post-transplant outcomes. Data collection was carried out by the SAAWP of the EBMT Data Office in Leiden, The Netherlands, according to EBMT guidelines. Letter invitations were sent to all SAAWP-affiliated centers by the EBMT data office. The EBMT office pre-filled the study report forms using data already available in the registry both for transplanted and non-transplanted patients; local investigators were asked to perform quality control and to provide additional information using the specific eCRF. After data collection, an extensive data quality check was performed, and in case of any discrepancy, additional queries were sent directly to the investigators involved in the study.

The final analysis was performed by the EBMT statistical office in Leiden after data collection and quality check.

Statistical analysis

Median values and interquartile ranges (IQR) were used to describe continuous variables, and frequencies and percentages were used to summarize categorical variables.

Probabilities of survival for overall survival (OS), failure-free survival (FFS), and transplant-free survival (TFS) were calculated using Kaplan-Meier estimates, with differences between the curves based on log-rank tests. OS was defined as the time from first androgen use to death from any cause. FFS was defined as the time from first androgen use to the introduction of a further treatment line, HCT, or death. TFS was defined as the time from first androgen use to HCT or death. In the case of non-event, observations were censored at the time of last follow-up or by five years after the start of follow-up, whichever came first.

Secondary analyzed endpoints included: cumulative incidences (CI) of relapse, clonal evolution, and toxicity events, calculated in a competing risk setting, where death before relapse or next treatment were considered the competing events. Competing risks analyses were also applied to post transplant outcomes of acute graft-*versus*-host disease (GvHD) grade II-IV and chronic GvHD, where only death was considered a competing event. Multivariable Cox proportional hazards models of FFS were constructed separately in the acquired and inherited BMF cohorts, with variables selected for their clinical significance.

All statistical tests were two-sided, and $P<0.05$ was considered statistically significant.

Statistical analyses were performed with R-Project 4.0 software packages (R Foundation for Statistical Computing, Vienna, Austria).

Results

Clinical landscape of androgen use in bone marrow failure

First, we sought to provide an epidemiologic picture of the use of androgens in EBMT-affiliated centers to understand their indications, patient demographics, disease context, treatment associations and their hierarchical place in the BMF therapeutic landscape. Overall, among 13,239 patients reported in the SAAWP registry, we identified 1,198 patients receiving an androgen treatment. We restricted our analysis to those who were treated before or in the absence of transplant between 1997 and 2021, and without missing status at the last follow-up. We thus selected 274 eligible patients, across 82 centers: 193 with acquired and 81 with inherited BMF. (See the Consort diagram in *Online Supplementary Figure S1* and patients' characteristics in Table 1).

In acquired BMF, the principal diagnosis was idiopathic aplastic anemia (AA; n=176, 91%), followed by pure red cell aplasia (PRCA; n=6, 3%), hemolytic paroxysmal nocturnal hemoglobinuria (PNH; n=6, 3%), and other acquired cytopenic syndromes (n=5, 3%) (Figure 1A). Most cases with idiopathic BMF, had severe/very severe AA at diagnosis (Figure 1B). Median age at the time of androgen initiation was 32 years (IQR: 18-52) and 65% of patients were male. Median time from diagnosis to first androgen use was 4 months (IQR: 0.3-17.6), with 54% of patients receiving androgens frontline and often associated with immunosuppressive treatments (IST) (Figure 1C). In this group, these compounds were administered as second-line, third-line, or further treatment in 23%, 13%, and 10% of the patients, respectively. In patients with available data, oxymetholone was the most common anabolic steroid used in acquired BMF (57%), followed by danazol (30%), testosterone (5%), norethandrolone (4%), and others (2%) (Figure 1D). Interestingly, androgen use across the participating centers varied, with a major tendency for their upfront use in acquired BMF in centers where ATG was not available (*Online*

Table 1. Patient characteristics.

		Acquired			Inherited		
		N/median	%/IQR	Missing, N	N/median	%/IQR	Missing, N
Total		193	100	-	81	100	-
Severity of aplastic anemia	Moderate	39	26	45	2	22	72
	Severe	74	50		6	67	
	Very severe	35	24		1	11	
Sex	Male	125	65	-	41	52	-
	Female	68	35	-	38	48	-
Age at diagnosis in years		32.2	17.1-52.3	-	6.5	4.1-9.3	-
Hemoglobin at diagnosis, g/dL		7.9	6.2-9.4	50	9.1	7.7-11.2	31
Neutrophils at diagnosis, x10^9/L		0.8	0.3-1.2	51	1	0.8-1.9	37
Platelets at diagnosis, x10^9/L		12	6-24	49	41	16-63.2	33
Age at androgen treatment for inherited group in years		32.9	18.7-52.6	-	6.5	4.1-9.3	-
Interval from diagnosis to androgen treatment in months		3.7	0.3-17.6	-	8.5	0.3-35.6	-
Reticulocytes on first androgen treatment, x10^9/L		41	18-60	156	37	11-59	66
Neutrophils on first androgen treatment, x10^9/L		1	0.6-1.6	111	0.8	0.4-1.3	50
Platelets on first androgen treatment, x10^9/L		18	6-31	108	28	13.5-39	47
N of RBC transfusions on 1st androgen treatment	<20 units	57	56	92	27	49	26
	20-50 units	23	23		8	14	
	>50 units	10	10		2	4	
	None	11	11		18	33	
N of platelet transfusions on 1st androgen treatment	<20 units	59	59	93	20	37	27
	20-50 units	18	18		7	13	
	>50 units	8	8		5	9	
	None	15	15		22	41	
N of lines before androgens	0	105	54	-	74	91	-
	1	44	23		6	7	
	2	25	13		1	1	
	>2	19	10		-	-	
Type of androgen	Danazol	34	30	81	12	36	48
	Nandrolone	1	1		1	3	
	Oxymetholone	64	57		6	19	
	Other	2	2		2	6	
	Nilevar	5	4		12	36	
	Testosterone	6	5		-	-	
Duration 1st androgen treatment in months		5.6	2.2-20.4	90	20	7-37.7	-
Concomitant growth factors	Yes	11	10	81	5	16	50
	No	101	90		26	84	
Concomitant association with IST	Yes	80	71	80	7	22	47
	No	33	29		25	78	
Type of IST used	CNI-based associations	29	20	47	3	50	1
	ATG-based associations	2	1		-	-	
	Other	3	2		3	50	

IQR: interquartile range; N: number; RBC: red blood cell count; IST: immunosuppressive therapy; CNI: calcineurin inhibitor; ATG: anti-thymocyte globulin.

Supplementary Table S1, Online Supplementary Figure S2A, B) and, in general, for patients not eligible for transplant or intensive IST (84%).

In inherited BMF, most of the patients were diagnosed with Fanconi anemia (FA; n=70, 86%), followed by Dyskeratosis congenita (DC; n=9, 11%), and other BMF disorders (n=2, 3%) (Figure 1A). Also in this group, severe/very severe BMF was the predominant phenotype at diagnosis (Figure 1B); 52% of patients were male. Median age at the time of androgen start was 8 years (IQR: 6-12), while median time from diagnosis to first androgen use was 8.5 months (IQR: 0.4-34.9), with androgen given as first-line treatment in 91% of cases. Frequently-used compounds were norethandrolone (36%) and danazol (36%), with a minority of patients receiving oxymetholone (19%), nandrolone (3%), and other androgens (6%) (Figure 1D).

Response and clinical outcomes after androgen therapy in acquired bone marrow failure

Median duration of androgen treatment in patients with acquired BMF was 5.6 months (range: 2.2-20.4) months. After three months of treatment, complete (CR) and partial (PR) remission were observed in 6% and 29% of patients, respectively, with most of the patients remaining in stable disease (SD) (Figure 2A). With a median follow-up from androgen initiation of 73.7 months (IQR: 57.1-96.4),

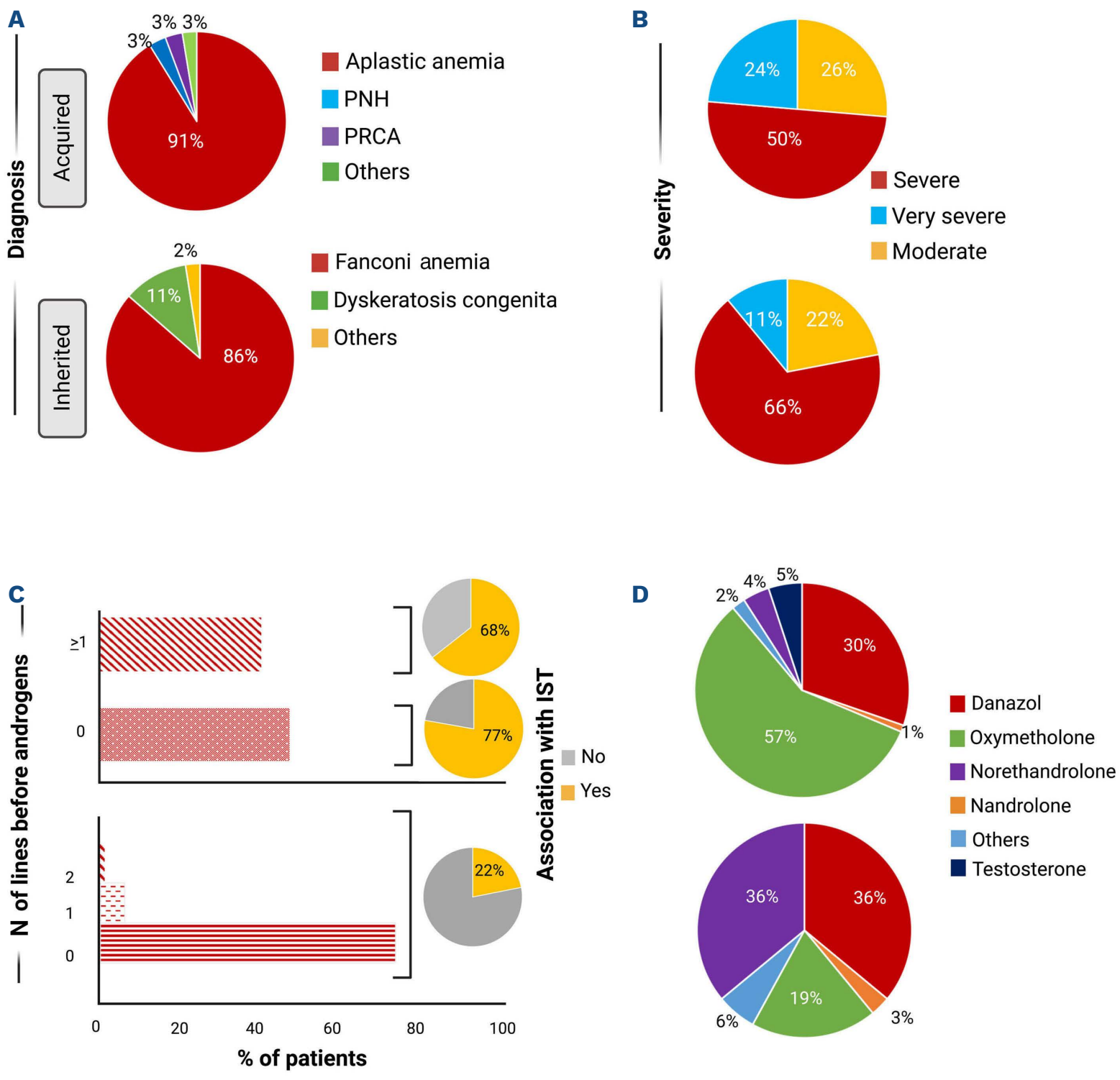

Figure 1. Clinical background of acquired and inherited bone marrow failure cohorts. Pie charts showing data related to acquired (top) and inherited (bottom) bone marrow failure (BMF) disorders. (A) Diagnosis as identified in the registry. (B) Distribution of severity of BMF. (C) Bar charts showing the distribution of patients according to the number of lines received before androgen therapy. Pie charts displaying the associations with immunosuppressive therapy (IST) (top: acquired BMF; bottom: inherited BMF). (D) Pie charts showing the distribution of androgenic compounds used in acquired (top) and inherited (bottom) BMF. N: number; PNH: paroxysmal nocturnal hemoglobinuria; PRCA: pure red cell aplasia.

5-year OS was 63% (95% Confidence Interval [CI]: 56-71%), TFS was 36% (28-43%), and FFS was 23% (16-30%) (Figure 2B-D). Five-year CI of relapse was 3% (0-6%). In an attempt to understand the factors associated with survival outcomes after androgen treatment, we next performed a univariable analysis of the weight of several co-variates on OS and FFS. We found that patients starting androgens more than 12 months after diagnosis, as well as patients given this treatment beyond second line, had better OS and FFS. Age showed an impact only on OS but not on FFS, with adult patients experiencing higher survival probabilities; sex did not impact on outcomes (*Online Supplementary Table S2, Online Supplementary Figure S3A-D*). Year of treatment was shown to impact OS but not FFS, with better survival outcomes in patients treated after 2010 (*Online Supplementary Figure S4A, B*). When multivariable models were fitted, the use of androgen after the second line of treatment remained the only factor positively impacting FFS (Hazard ration [HR] 0.32; [95% CI: 0.15-0.67]; *P*<0.001) (Figure 4A).

Response and clinical outcomes after androgen therapy in inherited bone marrow failure

In inherited BMF, androgens were given for a median duration of 20 months (IQR: 7-37.7).

After androgen start, CR and PR rates were observed respectively in 8% and 29% of patients at 3 months, while at 6 months we recorded 45% of PR without any patient achieving CR (Figure 3A). Considering the diagnostic subgroups, all DC patients with available response information (n=4) remained in SD at three months, with one subject reaching a PR at six months. Of note, the proportion of PR and CR were higher in the FA group although there were no significant differences between the two inherited subgroups at three (*P*=0.25) and six (*P*=0.73) months. For all patients with inherited BMF, median follow-up from androgen start was 82.3 (65.3-120.2) months, with 5-year OS, TFS, and FFS of 78% (68-87%), 17% (9-26%), and 14% (6-22%), respectively (Figure 3B-D). Univariable analyses showed no impact of any of the aforementioned factors (sex, age at androgen treatment,

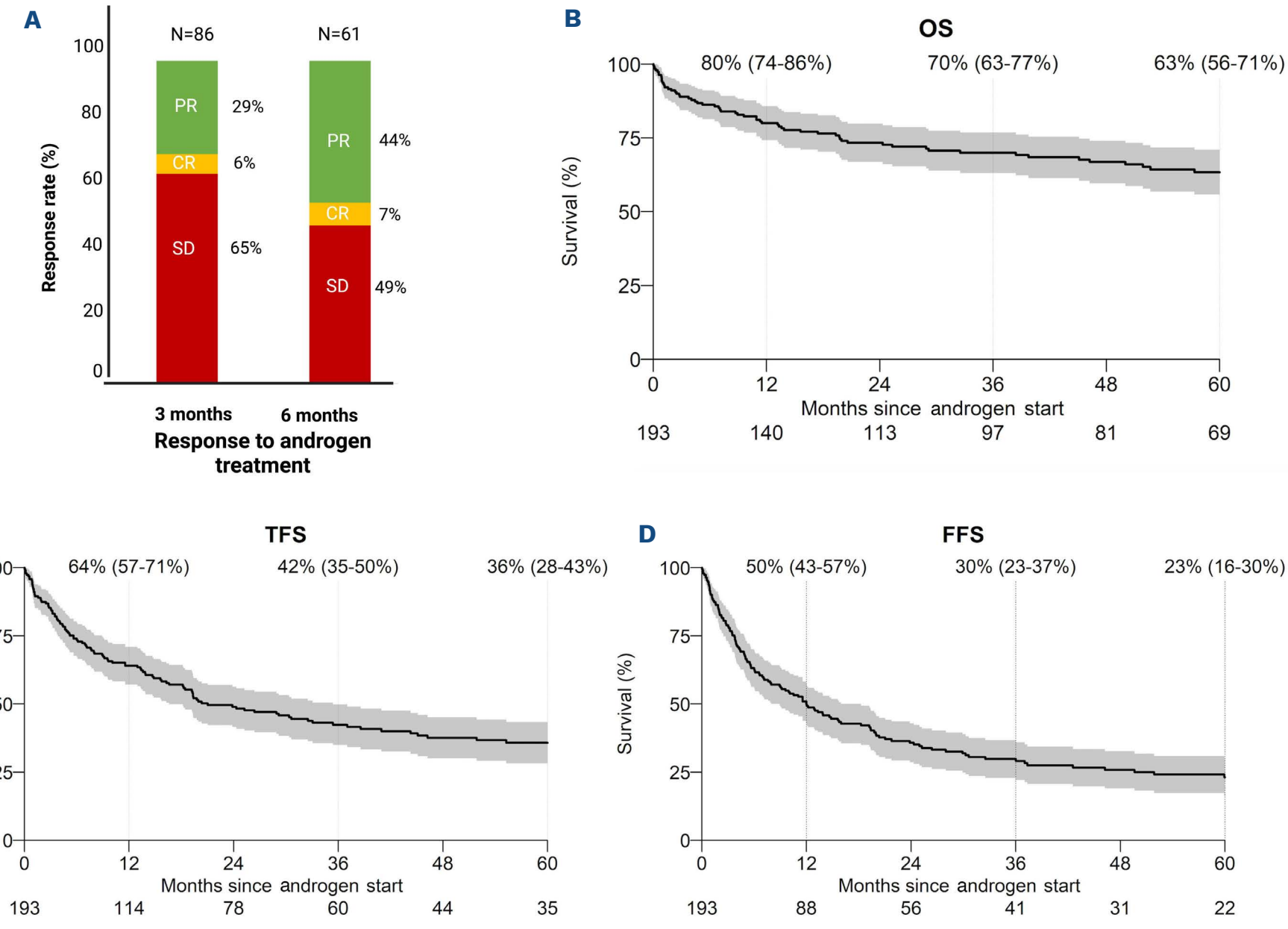

Figure 2. Clinical outcomes of acquired bone marrow failure patients. (A) Bar chart displaying the response rates of patients with acquired disorders. PR: partial remission; CR: complete remission; SD: stable disease; N: total number of patients with complete information for the analysis of response (at 3 months and 6 months after androgen start). Percentages show response rate. Kaplan Meyer estimates of (B) overall survival (OS), (C) transplant-free survival (TFS), and (D) failure-free survival (FFS).

interval from diagnosis, androgen line) on either OS or FFS (*Online Supplementary Figure S5A-D*). However, a detrimental impact of year of treatment was observed for FFS, with patients treated with androgens after 2010 showing lower probabilities of survival (*Online Supplementary Figure S6A, B*). Nevertheless, in multivariable analysis, a longer time from diagnosis to androgen start contributed to better FFS (Figure 4B).

Clonal evolution and androgen-related toxicity

With regards to the clonal evolution events, we observed a 5-year CI of acute myeloid leukemia and myelodysplastic syndromes (AML/MDS) of 3% (0-5%) in acquired and of 8% (0-12%) in inherited BMF, while probability of developing PNH was 2% (0-5%) in acquired disorders. All these events were considered in a competing risk setting before transplant and before other therapies. As to the rate of secondary neoplasms after androgenic treatment, we observed a 5-year CI of 1% (95% CI: 0-4%) in both groups.

To examine the probability of specific organ-related toxicity after androgen treatment, we collected and analyzed early and late events attributed to this treatment in a competing risk setting. Overall, these data were available in 110 patients: 79 acquired and 31 inherited BMF. Interestingly, we observed that in acquired BMF, where these events were more frequent, treatment-related toxicity tended to mostly occur in early phases after androgens (median time to liver, gastrointestinal, and renal toxicity was 2.8, 6.3, 5.4 months post treatment, respectively) (*Online Supplementary Table S3*), with only one patient experiencing psychiatric effects appearing after 18 months. In acquired BMF, the 5-year CI was 13% (95% CI: 6-19%) for liver, 4% (95% CI: 0-8%) for gastrointestinal, 3% (95% CI: 0-6%) for renal, 1% (95% CI: 0-3%) for psychiatric disorders, with most events occurring within the first year.

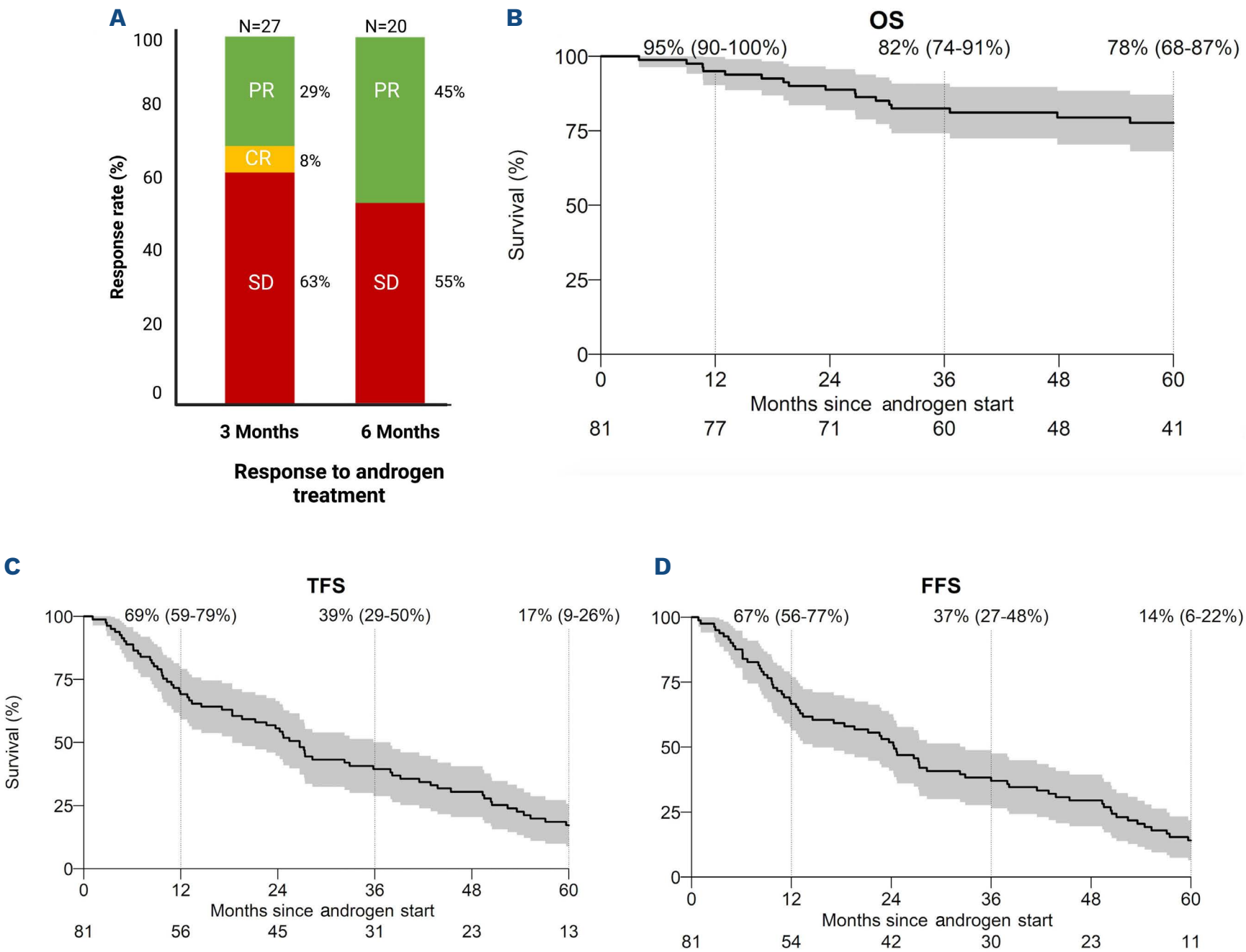

Figure 3. Clinical outcomes of inherited bone marrow failure patients. (A) Bar chart displaying the response rates of patients with inherited disorders. PR: partial remission; CR: complete remission; SD: stable disease; N: total number of patients with complete information for the analysis of response (at 3 months and 6 months after androgen start). Percentages show response rate. Kaplan Meyer estimates of (B) overall survival (OS), (C) transplant-free survival (TFS), and (D) failure-free survival (FFS).

For patients with inherited BMF, median time to toxicity from androgen initiation was more delayed: 22.8 (IQR: 8-41.5) and 15.1 (IQR: 11.5-18.7) months for liver and endocrinological toxicities, with a 5-year cumulative incidence of 13% (95% CI: 1-25%) and 6% (95% CI: 0-15%), respectively. No other types of organ-specific damage were reported.

Outcomes of patients receiving transplant after androgen treatment

A total of 82 (79 with any follow-up after transplant) in acquired and 78 (77 with any follow-up after transplant) in inherited BMF received an allogeneic HCT (*Online Supplementary Table S4*) at a median time of 14.3 months

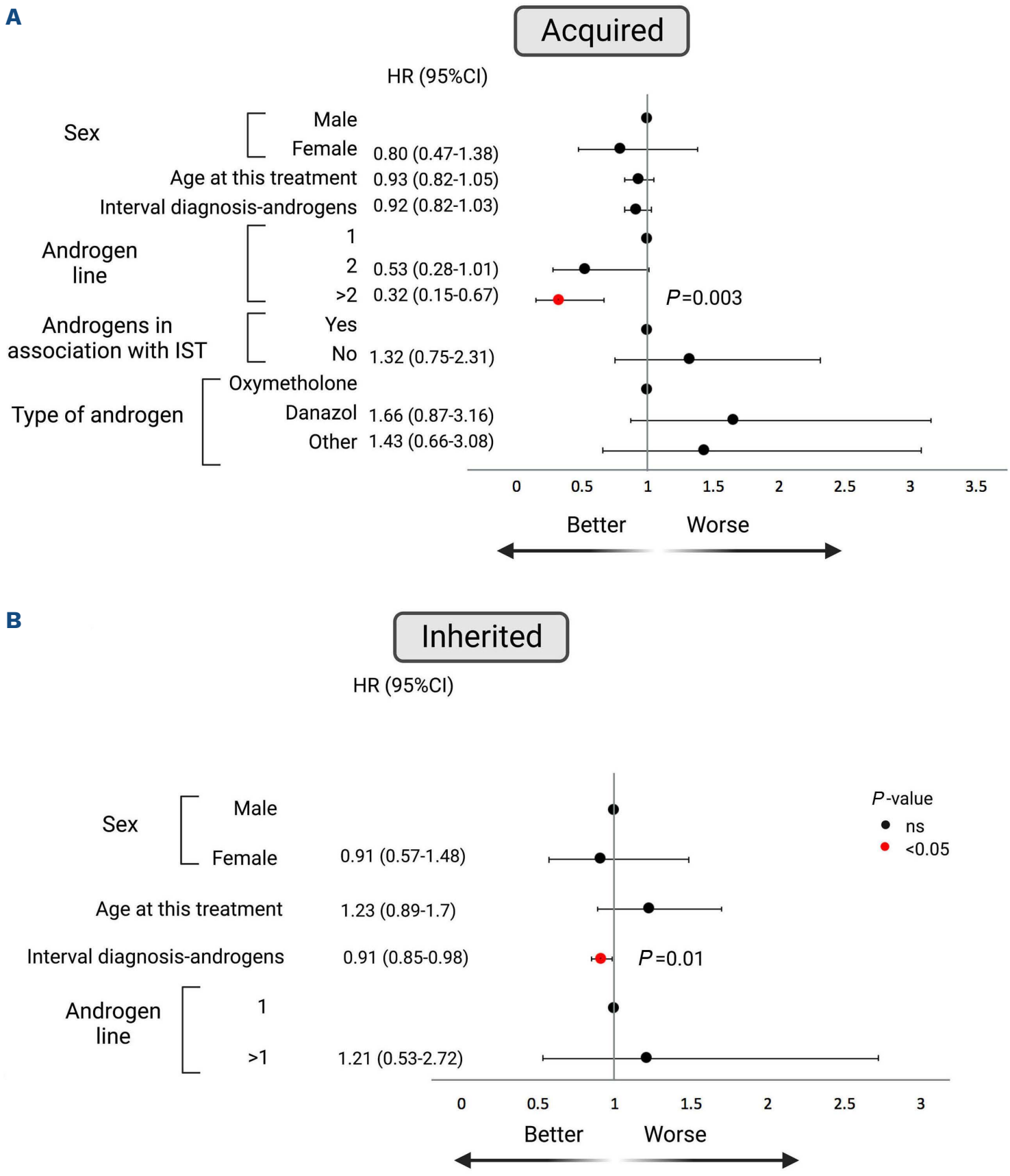

Figure 4. Multivariable cox regression analysis of failure-free survival. (A) Forest plot showing the Hazard Ratio (HR: black and red dots) and the 95% Confidence Intervals (CI: black horizontal lines) of each group of co-variates fitting into the model for failure-free survival (FFS) in patients with acquired bone marrow failure (BMF). (B) Forest plot showing the HR and the 95% CI of each group of co-variates fitting into the model for FFS in patients with inherited BMF. Of note, for both models only patients with complete co-variates, without missing status, were introduced into the models. The difference between the two models is principally due to the high number of missing data for the variables "Androgens in association with IST" (immunosuppressive therapy) and "Type of androgens" in the inherited group. For both panels, black dots indicate non-significant *P*-value; red dots: significant *P*-value. ns: not significant.

(IQR: 4.9-32.4) and 25.1 months (9.9-50.5) after androgen start, respectively. For patients with acquired BMF, median follow-up after transplant was 92.2 months (95% CI: 83.7-118.9) with a 5-year OS of 71% (61-81%). In inherited BMF, with a median follow-up of a median 56.8 months (95% CI: 36.2-68.5), 5-year OS was 57% (45-69%) (*Online Supplementary Figure S7A, B*).

The CI of day 100 acute GvHD grades II-IV was 18% (95% CI: 9-27%) in acquired and 30% (95% CI: 20-41%) in inherited BMF (*Online Supplementary Figure S7C, D*), while 5-year CI of chronic GvHD was 25% (95% CI: 15-35%) and 27% (95% CI: 16-37%), respectively, with most of the events occurring within the first three years (*Online Supplementary Figure S7E, F*). Post-transplant relapse of the underlying BMF was estimated at 6% (95% CI: 0-11% and 0-13%) (*Online Supplementary Figure S7G, H*) for both groups, whereas no events of clonal evolution were observed after HCT in patients receiving androgen treatment.

Discussion

Although present in the therapeutic armamentarium of BMF syndromes since almost a century prior to ATG, in the current era, the role of anabolic steroids in the treatment algorithm of acquired and inherited aplastic disorders is controversial. This is due to the lack of prospective data on large cohorts of homogeneously treated patients and the decline in interest for these compounds after the approval of more efficacious treatments, especially in the acquired setting. Here, taking advantage of a disease-specific database and with the collaboration of referral centers worldwide, we were able to build, across a median follow-up period of almost eight years, the largest cohort of patients treated with androgens, re-evaluating their role in these disorders.

In patients with acquired BMF, we tracked two patterns of usage. A first tendency was to choose androgens as a first-line treatment, usually in combination with classical immunosuppressive agents. Such usage was specifically observed in countries with limited access to ATG and/or for patients without a suitable donor or not fit for transplant. Survival and failure outcomes remained very unfavorable for patients in this treatment category. A second and more frequent pattern concerned their use in refractory contexts, as a single or combination treatment. Although both scenarios were associated with low response rates, we observed a higher likelihood of FFS both in univariable and multivariable analyses, when androgens were given beyond the second line, and after >12 months from diagnosis. Interestingly the type of androgen did not seem to influence outcomes, at least in the acquired group, where a proper model could be constructed taking into consideration compounds more frequently reported. Furthermore, we were able to exclude any impact of sex and age on post-androgen outcomes, excluding a role of endogenous androgenic levels to influence outcome variability. Nevertheless, the status of missing data in key pre-treatment clinical and biological variables, including the associated treatments, the number of transfusions, the degree of cytopenia, the presence of a PNH clone, precludes the construction of complete models to assess the response. Also, it is important to point out that, because our study has been generated from an international registry, the criteria discriminating between inherited and acquired BMF, as well as the genes profiled during the diagnostic phase, were heterogeneous and thus may have introduced some bias. Despite these limitations, this study allows a real-life assessment of the use of these compounds and their impact on outcomes across multiple countries.

Results shown in our retrospective cohort, particularly in the inherited context, were unable to confirm the higher response rates observed in previous studies.[6,36,27,36] This may be due to the variety of compounds used, likely different from the ones previously investigated, but also to the high proportion of transplanted patients in our cohort. In our analysis, allo-HCT was used after failure of androgen treatment, justifying the low FFS seen in both groups. In patients with inherited disorders, these observations were possibly in line with the tendency to use androgens as a "bridge" to transplant while a suitable donor is identified. In the acquired context, the use as both first and further lines makes the scenario more complex, but, in general, the failure of previous treatments seems to be a factor associated with better response. Nonetheless, it is not unlikely that the higher FFS in these patients is possibly related to misdiagnosed inherited conditions, rather than the positive effect of androgens, particularly when diagnoses were assessed before the availability of genomic platforms. In addition, acquired BMF patients surviving >12 months could either have non-severe disease or some improvement in neutrophils, and this would impact the response to androgens used beyond second-line therapy.

In both acquired and inherited BMF, data concerning their toxicity profile shows a low incidence of secondary events, with low rates of associated solid or hematologic malignancies within a >5-year follow-up. This result was in contrast with the putative oncogenicity of these molecules, shown in *in vitro* and *in vivo* models, and needs to be confirmed with longer follow-up.[39-41] A possible explanation for this is the relatively low duration of androgen treatment in both of our cohorts, endorsed by a high failure rate. Nonetheless, one could speculate that the boundary between the absolute role of exogenous androgens in carcinogenesis and the intrinsic genetic predisposition to cancers of certain inherited disorders,

including FA and DC, so far remains hard to disentangle. Moreover, the CI of AML/MDS evolution does not seem to differ from the rates observed in the general population of AA patients in independent cohorts.[42]

Clinical trials are currently recruiting in the United States and France (clinicaltrials.gov identifiers: NCT03312400, NCT03312400) to assess the role of danazol in telomere disorders, with regard to its optimal dose, safety, effectiveness, and long-term effects, highlighting the urgent need of prospective data to answer these still open questions. A single arm prospective study from the Brazilian group recently showed an interest in intramuscular nandrolone decanoate administered every 15 days for two years in 17 patients with telomere biology disorders. The study demonstrated telomer elongation in 77% of the cases and response rates superior to 50% at three months post treatment, albeit with a high frequency of low-grade adverse events (mostly mild liver dysfunction, virilization, and acne).[43] Although our analysis did not show the same response rates and the same incidence of adverse events in patients with inherited disorders, including DC, we must acknowledge the limitations of our study, due to the small sample size of this particular subgroup and the heterogeneity of the treatments received. Nevertheless, we seek to highlight, in line with previous literature, the interest in the use of androgens in telomere disorders and FA patients, especially in the absence of a transplant indication or as a "bridge" to transplant.

In acquired BMF, no recent prospective trial has been conducted to evaluate the use of androgens, besides the historical aforementioned studies,[9,10] mostly investigating androgen-ATG combinations. It is, therefore, challenging to make robust recommendations for practising hematologists in this context. It is also worth mentioning the various issues concerning the reliability of the supply of some of these compounds in recent years (including danazol and norethandrolone, currently unavailable in several countries) that may have contributed to such a heterogeneous pattern of use. Nevertheless, based on these older references and our retrospective study, androgen administration should be discouraged as front-line therapy in the acquired setting but remains reasonable in other specific contexts. Although transplant procedures remain the gold standard when a suitable donor is available, in the case of younger patients, androgen treatment could still represent a possible "bridge" to transplant. It could also provide a solution in the case of donor unavailability, especially for older subjects, and in cases in which patients have already failed first-line IST, particularly in the era of upfront triple therapy.[44]

Older female patients with idiopathic AA and low neutrophil count were reported to have satisfactory responses to upfront androgen/IST treatment.[10] Our study did not show a gender-related impact on clinical outcomes, but this effect could be skewed by the heterogeneity of our cohort. While proper comparisons with control groups (e.g., patients not under androgen treatment) are not available in the present analysis, pre-transplant androgen exposure did not modify post-transplant outcomes.

Although a more granular genetic assessment could not be covered by our study, we recognize that better clinico-biological and molecular diagnostics are necessary to improve our capacity to identify patients who can actually benefit from androgens, especially for inherited disorders, through a personalized therapeutical approach, for example, identifying specific gene mutations able to increase patient sensitivity to these compounds. This would require ongoing enrollment into international registries to ensure that good-quality data could be captured in the next ten years.

In conclusion, although a clear picture will only be possible after prospective trials, this study provides a unique opportunity to re-examine the role of androgens in BMF, showing interesting outcomes if given after second-line treatments in acquired disorders or after 12 months from diagnosis in inherited syndromes, with reasonable toxicity rates, and no consequences on a possible later allogeneic HCT.

Disclosures

No conflicts of interest to disclose.

Contributions

SP and AK were Principal Investigators of the study, conceptualized the study design and data collection, and interpreted the data analysis. SP provided the study synopsis and the electronic clinical report form, wrote the manuscript, and designed the figures. DJE performed the statistical analysis. BP performed the data collection and co-ordinated the study at the EBMT level. RPL and AMR supervised the study, interpreted the data analysis, gave important intellectual input, and edited the manuscript. All other authors provided clinical and biological data of the patients enrolled in this study and contributed to patient management and recruitment. The EBMT Severe Aplastic Anemia Working Party data management team takes the responsibility for the integrity and accuracy of the data presented. All authors reviewed and approved the final version of this manuscript for publication.

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Data-sharing statement

All data supporting the findings of this study are available within the Article and in the Online Supplementary Appen-

dix. Patient clinical and biological data are intellectual properties of the European Society for Blood and Marrow Transplantation (EBMT) and of the centers involved in the study, but can be requested from the chair of the EBMT Severe Aplastic Anemia Working Party (SAAWP) and data management team: regis.peffaultdelatour@aphp.fr; amrisita@unina.it; saawpebmt@lumc.nl.

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