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

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

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Key points:

- We assembled a large international retrospective cohort of patients receiving androgens for bone marrow failure (BMF) disorders providing clinical and epidemiologic data concerning their use, efficacy and toxicities on behalf of SAAWP of EBMT.
- Although still occupying a place in the therapeutic scenario of BMF disorders androgen use is associated with poor outcomes and high incidence of failure.
- Initiation after at least two lines of treatment for acquired BMF, and a delay of more than 12 months from diagnosis for inherited BMF were identified as factors associated with improved failure free survival.

Running title: Androgens in BMF disorders

Supplementary appendix:

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AUTHORSHIP AND DISCLOSURES

Authorship contributions:

SP and AK were PIs of the study, conceptualized study design and data collection, and interpreted data analysis. SP provided the study synopsis and the eCRF, wrote the manuscript and designed the figures. DJE performed the statistical analysis. BP performed the data-collection and coordinated the study at the EBMT level. RPL and AMR supervised the study, interpreted the data analysis, gave important intellectual inputs 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. SAAWP 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.

Conflict-of-interest disclosure:

This research was conducted in absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data sharing

All the data that support the findings of this study are available within the Article and Supplementary Files. Patient level clinical and biological data are intellectual properties of EBMT and of the centers involved in the study but can be requested to the SAAWP chair and data management team (regis.peffaultdelatour@aphp.fr; amrisita@unina.it; saawpebmt@lumc.nl).

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Abstract

Androgens have represented the historical therapeutic backbone of bone marrow failure (BMF) syndromes. However, their role has been rarely analyzed in prospective setting and systematic and long-term data are currently unavailable regarding their usage, effectiveness and toxicity in both acquired and inherited BMF.

Here, taking advantage of a unique disease-specific international dataset, we retrospectively analyzed the so far largest cohort of BMF patients who received androgens before or in absence of an allogeneic hematopoietic cell transplantation (HCT), reappraising their current use in these disorders. We identified 274 patients across 82 EBMT affiliated centers, 193 with acquired (median age of 32) and 81 with inherited BMF (median age of 8 years). With a median duration of androgen treatment of 5.6 and 20 months respectively, complete/partial remission rates at 3 months were of 6%/29% in acquired and 8%/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 contexts. Androgen initiation after second line treatments for acquired, and after > 12 months post-diagnosis for inherited group 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 hematological 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 their use on behalf of the SAAWP 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 to the beginning of 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 hematological 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 immune suppressive regimens for the treatment of acquired or inherited BMF,[5,6,7,8] with data generated from randomized trials since early 80ies. [9,10] Specifically the discordant results produced by these historical trials, showing in one case no benefits in adding androgens to anti-thymoglobulin (ATG)[9] and in another case the superiority of the association in response but not in overall survival,[10] failed to prompt androgens as a front line treatment in 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, hemopoietic and immune system. [1,11,12,13]

Effects on hematopoiesis in the context of BMF may depend on pleiotropic mechanisms, including I) stimulation of erythropoietin (EPO) receptor expression on erythroid progenitors and increased iron mobilization via hepcidin inhibition;[14–16] II) telomere elongation in hematopoietic stem cells (HSCs) via binding to the estrogen response elements (EREs) present in *TERT* gene promoter after estrogen aromatization, indirectly impacting cell survival and proliferation signalling;[6,8,17–19,20] III) 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 of the observation of hematological

improvement under different androgenic analogues, the mechanism of specific molecules remains poorly understood. For instance, danazol has been reported as able to induce telomere elongation[6,8,17] despite it structurally being a non-aromatizable steroid, thus unable to interact directly via EREs in *TERT* gene promoter.[24,25] Biological consequences of the AR stimulation on hematopoiesis have been mostly 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.[7,17,26,27,28] In acquired setting, they have been used prior to the availability of anti-thymocyte globulin (ATG) and later on, in combination to it as front-line or subsequent therapeutic approaches.[5,9,10,29–34]

Danazol, 3-alpha-etiocholanolone, nandrolone, oxymetholone, oxandrolone are some of the anabolic-androgenic compounds used in BMF setting, showing variable response and toxicity profiles.[6,8,19,29–31,33,35] Although integrated in the therapeutic armamentarium for acquired and inherited BMF, many outstanding questions, as to the ideal molecule, optimal dose, timing, disease subgroup, place in the treatment algorithm, factors influencing response, remain unanswered. Moreover, lack of systematic analysis of long-term outcomes of patients receiving androgens, preclude clear positions on 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 accepted to participate to clinical and

biological research studies conducted on behalf of 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 anabolic steroids' usage from 1997 to 2021 as any line before or in absence of hematopoietic cell transplantation (HCT).

Primary objective was to assess the response to androgens in patients diagnosed with acquired and inherited BMFs. Secondary aims were to describe the background use of androgens, to assess clinical outcomes in patients receiving androgens including in transplant setting, to determine prognostic factors associated with failure, 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, Turkey.

An electronic clinical report form (eCRF) was sent to all participating centers with the aim 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 performed by the SAAWP of the EBMT Data Office in Leiden, Netherland, according to EBMT guidelines. Letter invitations were sent to all SAAWP-affiliated centers by the EBMT data office. 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 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.

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 5 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 GvHD grade II-IV and chronic GvHD, where only death was considered competing. 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 a P-value <0.05 was considered statistically significant.

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

Results

Clinical landscape of androgen use in BMF

Firstly, 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 therapeutic landscape. Overall, we identified among 13239 patients reported in SAAWP registry 1198 patients receiving an

androgen treatment. We restricted our analysis to those who were treated before or in 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 (Consort diagram in **Figure S1**, and patient characteristics in **table 1**).

For acquired group principal diagnosis in acquired group was idiopathic aplastic anemia (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 (IQR: 18-52) years, with 65% being male. Median time from diagnosis to first androgen use was 4 (IQR: 0.3- 17.6) months, with 54% of patients receiving androgens frontline and often associated with immunosuppressive treatments (**Figure 1C**). In this group these compounds were administered as 2nd, 3rd or further line in 23%, 13% and 10% of the patients, respectively. In patients with available data, oxymetholone was the most common anabolic steroid used in acquired context (57%), followed by danazol (30%), testosterone (5%), norethandrolone (4%), and others (2%) (**Figure 1D**). Interestingly, androgens' usage across the participating centers varied, with a major tendency for their upfront use in acquired context, in centers where ATG was not available (**Figure S2A-B**) and in general for patients not eligible for transplant or intensive IST (84%).

In the inherited context, 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**) with 52% being male patients (52%). Median age at the time of androgen start was 8 (IQR: 6-12) years, while median time from diagnosis to first androgen use was 8.5 (IQR: 0.4-34.9) months, with androgen given as first line treatment in 91% of cases. Frequently used compounds were norethandrolone (36%) and danazol (36%), with a minor part of patients receiving oxymetholone (19%), nandrolone (3%) and other androgens (6%) (**Figure 1D**).

Response and clinical outcomes after androgen therapy in acquired BMF

Median duration of androgen treatment in patients with acquired BMF was 5.6 (2.2-20.4) months. After three months of treatment, complete (CR) and partial remission (PR) 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), at 5-years, OS was 63% (95% confident interval [CI] 56-71%), TFS was 36% (28-43%), FFS was 23% (16-30%), **figure 2B-D**. Five-year CI of relapse was 3% (0-6%). In the attempt to understand the factors associated with survival outcomes after androgen treatment, we next analyzed in univariable setting the weight of several covariates on OS and FFS. We found that patients initiating androgens more than 12 months after the 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, while sex did not impact on outcomes (**Figure S3A-D, Table S2**). Year of treatment was showed to impact OS but not FFS, with better survival outcomes in patients treated after 2010 (**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-value <0.001, **Figure 4A**).

Response and clinical outcomes after androgen therapy in inherited BMF

In inherited context 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**). Looking at diagnostic subgroups, while all DC patients with available response information (N=4) remained in SD at three months, with one subject reaching a PR at 6 months, proportion of PR and CR were higher in FA group but, yet without significant differences between the two inherited subgroups at 3 (p=0.25) and 6 months (p=0.73). 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, interval from diagnosis, androgen line) both on OS and FFS (**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 (**Figure S6A-B**). Nevertheless, in multivariable analysis, a longer time to initiate androgens from diagnosis contributed to better FFS (**Figure 4B**).

Clonal evolution and androgen related toxicity

Looking at 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 group, while probability of developing PNH was 2% (0-5%) in acquired group. All these events occurred 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.

Seeking at reporting the probability of specific organ-related toxicity after androgen treatment, we put efforts in collecting and analyzing early and late events attributed to this treatment in a competitive risk setting. Overall, these data were available in 110 patients; 79 (56%) acquired and 31 (37%) inherited cases. Interestingly, we observed that in acquired setting, where these events were more frequent, treatment-related toxicity tended to mostly occur in early phases after androgens (for liver, gastrointestinal and renal toxicity median time was respectively 2.8, 6.3, 5.4 months post-treatment**, Table S3**), with only 1 case experiencing psychiatric effects appearing after 18 months.

In the acquired group, 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. 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 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 group received an allogeneic HCT (**Table S4**), at a median

time of 14.3 months (IQR: 4.9-32.4) and 25.1 months (9.9-50.5) after androgen initiation respectively. For patients with acquired BMF, median follow-up after transplant was 92.2 (95% CI 83.7 - 118.9) months with a 5-year OS of 71% (61-81%). In inherited group, with a median follow-up of median 56.8 (95%CI 36.2 - 68.5) months, 5-year OS was 57% (45-69%) (**Figure S7 A-B**).

CI of day 100 acute GvHD II-IV was 18% (95%CI 9-27%) in acquired and 30% (95%CI 20-41%) in inherited (**Figure S7 C-D**) while 5-year CI of chronic GvHD was respectively 25% (95%CI 15-35%) and 27% (95%CI 16-37%), with most of the events occurring within the first 3 years (**Figure S7 E-F)**. Post-transplant relapse of the underlined BMF was estimated at 6% (95%CI 0-11% and 0-13%, **Figure S7 G-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 one century even prior to ATG, role of anabolic steroids in the current era is controversial in the treatment algorithm of acquired and inherited aplastic disorders, because of lack of prospective data on large cohorts of homogeneously treated patients and decline in the interest for these compounds after the approval of more efficacious treatments, especially in acquired setting. Here, taking advantage of a disease-specific database and of the connections among referral centers worldwide, we were able to build, across a median follow-up period of almost 8 years, the largest cohort of patients treated with androgens, reappraising their role in these disorders.

In patients with acquired BMF, we tracked two patterns of usage. A first tendency was to choose androgens, usually in combination with classical immunosuppressive agents, as a first-line treatment. This behavior 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 combinatorial treatment. Although both scenarios were associated with low response rates, we observed 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 acquired group, where a proper model could be constructed taking in consideration compounds more frequently reported. Furthermore, we were able to exclude an 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 the 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 in our study and thus may have introduce 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.[6,36,28,37] 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 suitable donor is identified. In acquired context, the use as both first and further lines complexifies the scenario 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, more inclined to the positive effect of androgens, particularly when diagnoses were assessed before the availability of genomic platforms. Additionally, acquired BMF patients surviving >12 months could have either have non-severe disease or some improvement in neutrophils, and hence impacting the response to androgen used beyond second line therapy.

Data concerning their toxicity profile shows in both categories a low incidence of secondary events, with low rates of associated solid or hematological malignancies within a >5 years follow-up. This result was in contrast with the putative oncogenicity of these molecules, shown in *invitro* and *invivo* models, and needs to be confirmed with longer follow-up.[38–42] A

possible explanation of that is the relatively low duration of androgen treatment in both of our cohorts, endorsed by high rate of failure. 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, remains so far hard to disentangle. Moreover, the CI of AML/MDS evolution does not seem different from the rates observed in the general population of AA patients in independent cohorts.[43]

Clinical trials are currently recruiting in United States, and France (NCT03312400, NCT03312400) to assess the role of danazol in telomere disorders, in 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 recent single arm prospective study from the Brazilian group recently showed the interest of intramuscular nandrolone decanoate administered every 15 days for two years, in 17 patients with telomere biology disorders, demonstrating telomer elongation in 77% of the cases and response rates superior to 50% from 3 months post-treatment albeit with high frequency of low-grade adverse events (mostly mild liver dysfunction, virilization and acne).[44] Although our analysis did not display 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 of androgens' use in telomere disorders and FA patients, especially in 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 tedious to make robust recommendations for practicing hematologists in this context. It is also worth mentioning the variable supply issues with some of these compounds in recent years (including danazol and norethandrolone, currently unavailable in several countries), that may contribute to such a heterogeneous pattern of use. Nevertheless, based on these older references and our retrospective study, androgen administration should be discouraged as frontline therapy in acquired setting but remains reasonable in other specific contexts. Although transplant procedures remain the gold standard when a suitable donor is available and in case of younger patients, androgen

treatment could still represent a possible "bridge to transplant" or a solution in case of donor unavailability especially for older subjects, and in case patients have already failed the first line of immune suppression, especially in the era of upfront triple therapy.[45]

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 likely 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 use 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 discriminate patients who can actually benefit from androgens especially for inherited disorders, through a personalized therapeutical approach, for instance identifying specific gene-mutations able to increase the sensitivity to these compounds. This would require ongoing enrollment into international registries, thus good quality data could be captured in the next 10 years.

In conclusion, although clear statements will be possible only after prospective trials, this study provides a unique opportunity to reexamine 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.

References

1. Eder IE, Culig Z, Putz T, Nessler-Menardi C, Bartsch G, Klocker H. Molecular Biology of the Androgen Receptor: From Molecular Understanding to the Clinic. Eur Urol. 2001;40(3):241-251.
2. Shahidi NT, Diamond LK. Testosterone-induced remission in aplastic anemia. AMA J Dis Child. 1959;98293-98302.
3. Shahidi NT. Androgens and erythropoiesis. N Engl J Med. 1973;289(2):72-80.
4. Kennedy BJ. EFFECTS OF INTENSIVE SEX STEROID HORMONE THERAPY IN ADVANCED BREAST CANCER. JAMA. 1953;152(12):1135.
5. Najean Y, Haguenauer O. Long-term (5 to 20 years) Evolution of nongrafted aplastic anemias. The Cooperative Group for the Study of Aplastic and Refractory Anemias. Blood. 1990;76(11):2222-2228.
6. Townsley DM, Dumitriu B, Liu D, et al. Danazol Treatment for Telomere Diseases. N Engl J Med. 2016;374(20):1922-1931.
7. Kirschner M, Vieri M, Kricheldorf K, et al. Androgen derivatives improve blood counts and elongate telomere length in adult cryptic dyskeratosis congenita. Br J Haematol. 2021;193(3):669-673.
8. Vieri M, Kirschner M, Tometten M, et al. Comparable Effects of the Androgen Derivatives Danazol, Oxymetholone and Nandrolone on Telomerase Activity in Human Primary Hematopoietic Cells from Patients with Dyskeratosis Congenita. Int J Mol Sci. 2020;21(19):E7196.
9. Champlin RE, Ho WG, Feig SA, Winston DJ, Lenarsky C, Gale RP. Do androgens enhance the response to antithymocyte globulin in patients with aplastic anemia? A prospective randomized trial. Blood. 1985;66(1):184-188.
10. Bacigalupo A, Chaple M, Hows J, et al. Treatment of aplastic anaemia (AA) with antilymphocyte globulin (ALG) and methylprednisolone (MPred) with or without androgens: a randomized trial from the EBMT SAA working party. Br J Haematol. 1993;83(1):145-151.
11. Davey RA, Grossmann M. Androgen Receptor Structure, Function and Biology: From Bench to Bedside. Clin Biochem Rev. 2016;37(1):3-15.
12. Papakonstanti EA, Kampa M, Castanas E, Stournaras C. A Rapid, Nongenomic, Signaling Pathway Regulates the Actin Reorganization Induced by Activation of Membrane Testosterone Receptors. Mol Endocrinol. 2003;17(5):870-881.
13. Estrada M, Espinosa A, Müller M, Jaimovich E. Testosterone Stimulates Intracellular Calcium Release and Mitogen-Activated Protein Kinases Via a G Protein-Coupled Receptor in Skeletal Muscle Cells. Endocrinology. 2003;144(8):3586-3597.
14. Bachman E, Feng R, Travison T, et al. Testosterone suppresses hepcidin in men: a potential mechanism for testosterone-induced erythrocytosis. J Clin Endocrinol Metab. 2010;95(10):4743-4747.
15. Guo W, Bachman E, Li M, et al. Testosterone administration inhibits hepcidin transcription and is associated with increased iron incorporation into red blood cells. Aging Cell. 2013;12(2):280-291.
16. Bachman E, Travison TG, Basaria S, et al. Testosterone induces erythrocytosis via increased erythropoietin and suppressed hepcidin: evidence for a new erythropoietin/hemoglobin set point. J Gerontol A Biol Sci Med Sci. 2014;69(6):725-735.
17. Calado RT, Yewdell WT, Wilkerson KL, et al. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. Blood. 2009;114(11):2236-2243.
18. Townsley DM, Dumitriu B, Young NS. Bone marrow failure and the telomeropathies. Blood. 2014;124(18):2775-2783.
19. Kirschner M, Vieri M, Kricheldorf K, et al. Androgen derivatives improve blood counts and elongate telomere length in adult cryptic dyskeratosis congenita. Br J Haematol. 2021;193(3):669-673.
20. Bar C, Huber N, Beier F, Blasco MA. Therapeutic effect of androgen therapy in a mouse model of aplastic anemia produced by short telomeres. Haematologica. 2015;100(10):1267-1274.
21. Radojević K, Arsenović-Ranin N, Kosec D, et al. Neonatal castration affects intrathymic kinetics of T-cell differentiation and the spleen T-cell level. J Endocrinol. 2007;192(3):669-682.
22. Pergola C, Dodt G, Rossi A, et al. ERK-mediated regulation of leukotriene biosynthesis by androgens: a molecular basis for gender differences in inflammation and asthma. Proc Natl Acad Sci U S A. 2008;105(50):19881-19886.
23. Guan X, Polesso F, Wang C, et al. Androgen receptor activity in T cells limits checkpoint blockade efficacy. Nature. 2022;606(7915):791-796.
24. Danazol Treatment for Telomere Diseases. N Engl J Med. 2016;375(11):1095-1096.
25. Deocaporto R, Fernandez A, Brito D, Vidal T, Diaz A. Gas chromatography/mass spectrometry characterization of urinary metabolites of danazol after oral administration in human. J Chromatogr B Analyt

Biomed Life Sci. 2006;830(1):178-183.
26. Scheckenbach K, Morgan M, Filger-Brillinger J, et al. Treatment of the bone marrow failure in Fanconi anemia patients with danazol. Blood Cells Mol Dis. 2012;48(2):128-131.
27. Scheckenbach K, Morgan M, Filger-Brillinger J, et al. Treatment of the bone marrow failure in Fanconi anemia patients with danazol. Blood Cells Mol Dis. 2012;48(2):128-131.
28. Paustian L, Chao MM, Hanenberg H, et al. Androgen therapy in Fanconi anemia: A retrospective analysis of 30 years in Germany. Pediatr Hematol Oncol. 2016;33(1):5-12.
29. Seewald TR, Zeigler ZR, Gardner FH. Successful treatment of severe refractory aplastic anemia with 3-beta etiocholanolone and nandrolone decanoate. Am J Hematol. 1989;31(3):216-218.
30. Gardner FH, Juneja HS. Androstane therapy to treat aplastic anaemia in adults: an uncontrolled pilot study. Br J Haematol. 1987;65(3):295-300.
31. Androgen therapy in aplastic anaemia: a comparative study of high and low-doses and of 4 different androgens. French Cooperative Group for the Study of Aplastic and Refractory Anemias. Scand J Haematol. 1986;36(4):346-352.
32. Camitta B, O'Reilly RJ, Sensenbrenner L, et al. Antithoracic duct lymphocyte globulin therapy of severe aplastic anemia. Blood. 1983;62(4):883-888.
33. Jaime-Pérez JC, Colunga-Pedraza PR, Gómez-Ramírez CD, et al. Danazol as first-line therapy for aplastic anemia. Ann Hematol. 2011;90(5):523-527.
34. Pierri F, Dufour C. Management of aplastic anemia after failure of frontline immunosuppression. Expert Rev Hematol. 2019;12(10):809-819.
35. Rose SR, Kim M-O, Korbee L, et al. Oxandrolone for the treatment of bone marrow failure in Fanconi anemia: Oxandrolone Use in FA Bone Marrow Failure. Pediat Blood Cancer. 2014;61(1):11-19.
36. Khincha PP, Wentzensen IM, Giri N, Alter BP, Savage SA. Response to androgen therapy in patients with dyskeratosis congenita. Br J Haematol. 2014;165(3):349-357.
37. Català A, Ali SS, Cuvelier GDE, et al. Androgen therapy in inherited bone marrow failure syndromes: analysis from the Canadian Inherited Marrow Failure Registry. Br J Haematol. 2020;189(5):976-981.
38. Liao DJ, Dickson RB. Roles of androgens in the development, growth, and carcinogenesis of the mammary gland. J Steroid Biochem Mol Biol. 2002;80(2):175-189.
39. Huang H, Zegarra-Moro OL, Benson D, Tindall DJ. Androgens repress Bcl-2 expression via activation of the retinoblastoma (RB) protein in prostate cancer cells. Oncogene. 2004;23(12):2161-2176.
40. Horning ES. Carcinogenic Action of Androgens. Br J Cancer. 1958;12(3):414-418.
41. Escrich E, Solanas M, Bailly C, Ruiz de Villa MC, Saez S. Effects of an androgenic derivative on the development of chemically-induced mammary carcinogenesis in the rat. Anticancer Res. 1994;14(2A):539-543.
42. Choi J, Psarommatis B, Gao YR, Zheng Y, Handelsman DJ, Simanainen U. The role of androgens in experimental rodent mammary carcinogenesis. Breast Cancer Res. 2014;16(6):483.
43. Gurnari C, Pagliuca S, Prata PH, et al. Clinical and Molecular Determinants of Clonal Evolution in Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria. J Clin Oncol. 2022;41(1):132-142.
44. Clé DV, Catto LFB, Gutierrez-Rodrigues F, et al. Effects of nandrolone decanoate on telomere length and clinical outcome in patients with telomeropathies: a prospective trial. Haematologica. 2023;108(5):1300-1312.
45. Peffault de Latour R, Kulasekararaj A, Iacobelli S, et al. Eltrombopag Added to Immunosuppression in Severe Aplastic Anemia. N Engl J Med. 2022;386(1):11-23.

Table 1: Patient characteristics at androgen treatment							
		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 (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 treatmant (years)		32.9	(18.7-52.6)		6.5	(4.1-9.3)	
Interval from diagnosis to Androgen treatment (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
Number of RBC transfusions on first 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	

Number of platelet transfusions on first 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	
Number 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 first androgen treatment (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	

Figure Legend

Figure 1: Clinical background of acquired and inherited BMF cohorts. A) Pie charts showing the distributions of diagnosis as identified into the registry in inherited and acquired disorders. **B)** Pie charts depicting the distribution of severity of BMF disorders (upper: acquired, lower: inherited). **C)** Bar charts showing the distribution of patients according the number of lines received before androgen therapy (upper: acquired, lower: inherited). Pie charts showing the associations with IST (gray: No; yellow: Yes; upper: acquired, lower: inherited). **D)** Pie charts showing the distribution of androgenic compounds used for acquired (upper) and inherited (lower) disorders.

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

Figure 3: Clinical outcomes of inherited BMF patients. A) Bar chart displaying the response rates of patients with inherited disorders. PR: partial remission, CR: complete remission, SD: stable disease. N indicates the total number of patients with complete information for the analysis of response (at 3 months and 6 months after androgen initiation). Percentages on the side show the response rate. **B)** Kaplan Meyer estimates of overall survival. **C)** Kaplan Meyer estimates of transplant free survival. **D)** Kaplan Meyer estimates of failure free survival.

Figure 4: Multivariable cox regression analysis of failure free survival (FFS). A) Forest plot showing the hazard ratio (HR, black and red dots) and the 95% confident intervals (CI, black horizontal lines) of each group of covariates fitting into the model for FFS in patients with acquired BMF. **B)** Forest plot showing the HR and the 95% CI of each group of covariates fitting into the model for FFS in patients with inherited BMF.
Of note for both models only patients with complete covariates, 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” and “Type of androgens” in inherited group. For both panels, black dots indicate non-significant p-value, red dots: significant p-value.

Figure 1

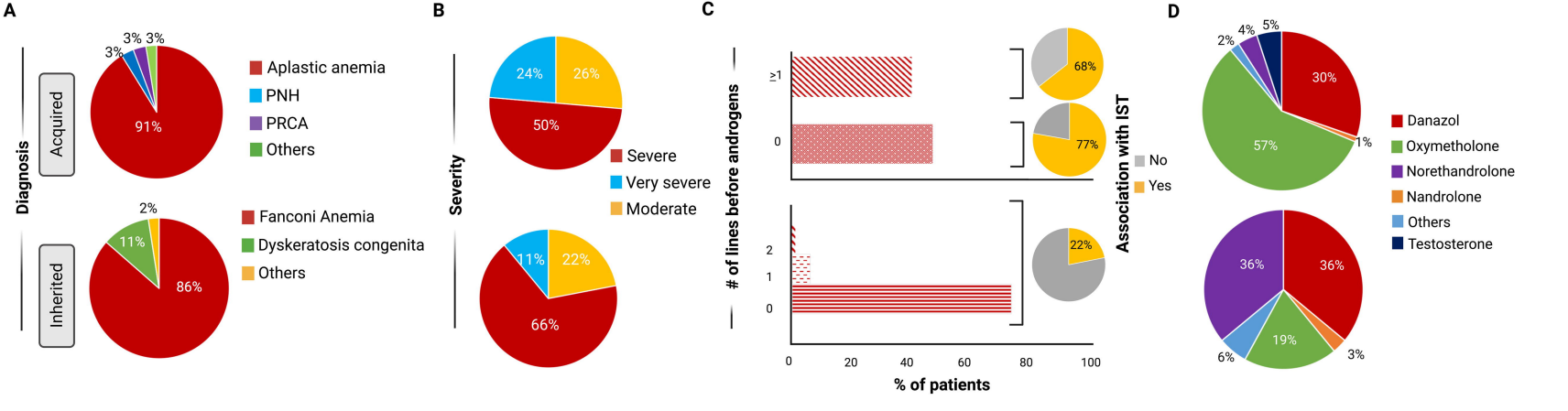

A
Diagnosis
Acquired
3%
3%
3%
91%
Aplastic anemia
PNH
PRCA
Others
Inherited
2%
11%
86%
Fanconi Anemia
Dyskeratosis congenita
Others
B
Severity
24%
26%
50%
Severe
Very severe
Moderate
11%
22%
66%
C
# of lines before androgens
≥1
0
2
1
0
68%
77%
22%
Association with IST
No
Yes
0
20
40
60
80
100
% of patients
D
2%
4%
5%
30%
1%
57%
36%
36%
19%
6%
3%
Danazol
Oxymetholone
Norethandrolone
Nandrolone
Others
Testosterone

Figure 2

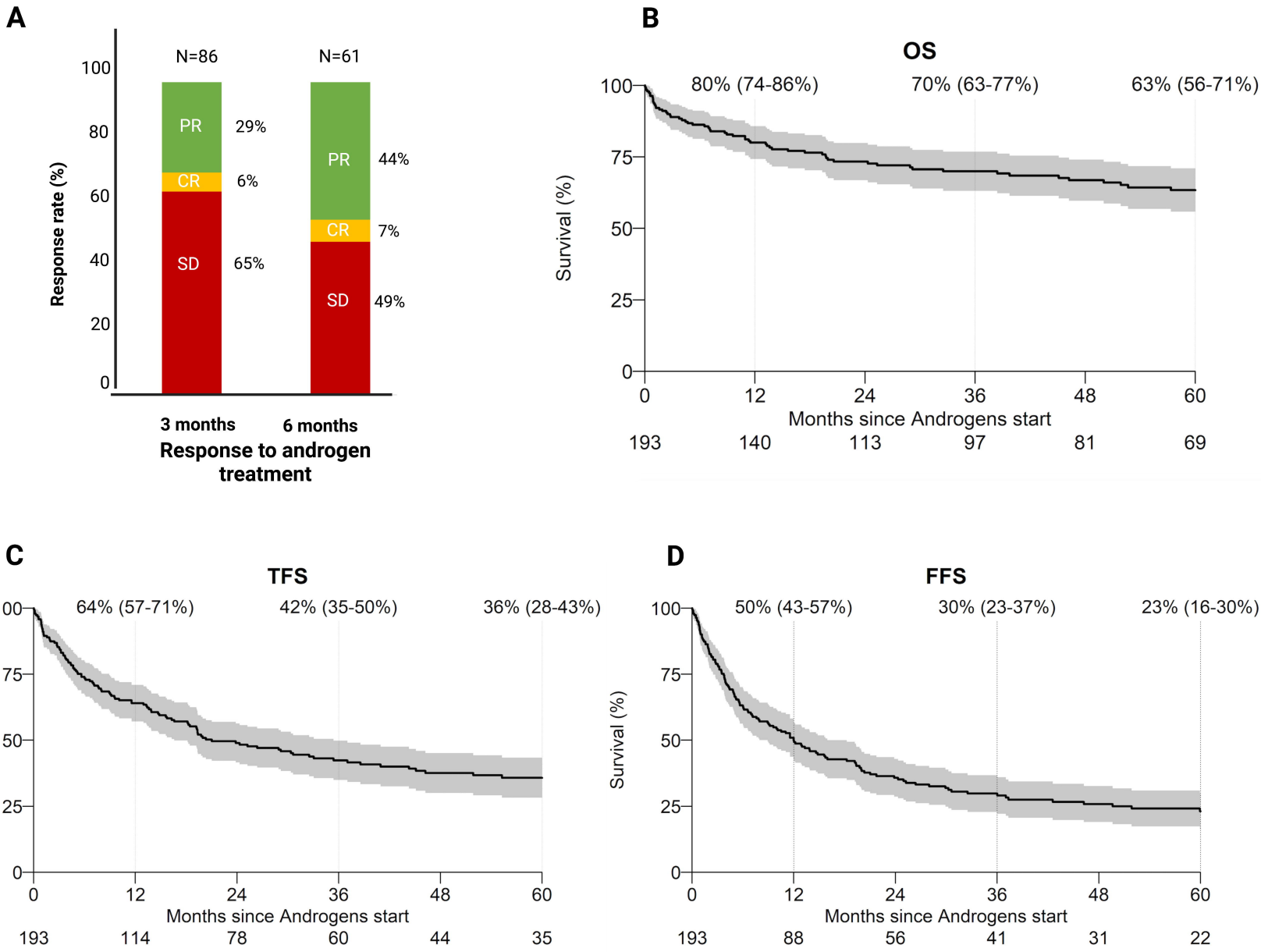

A
N=86
N=61
100
80
60
40
20
0
Response rate (%)
PR
29%
CR
6%
SD
65%
PR
44%
CR
7%
SD
49%
3 months
6 months
Response to androgen treatment
B
OS
80% (74-86%)
70% (63-77%)
63% (56-71%)
Survival (%)
Months since Androgens start
193
140
113
97
81
69
C
TFS
64% (57-71%)
42% (35-50%)
36% (28-43%)
Survival (%)
Months since Androgens start
193
114
78
60
44
35
D
FFS
50% (43-57%)
30% (23-37%)
23% (16-30%)
Survival (%)
Months since Androgens start
193
88
56
41
31
22

Figure 3

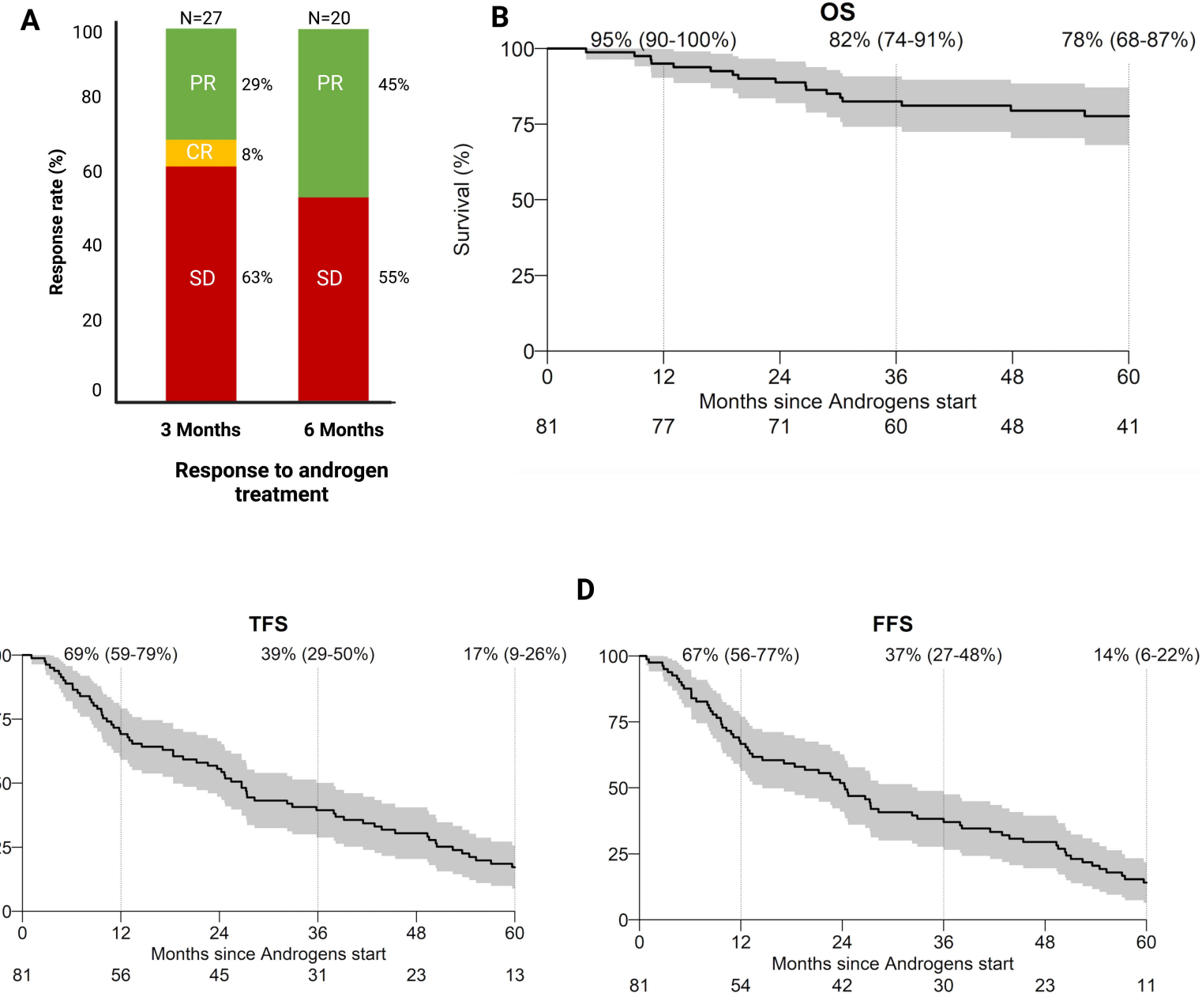
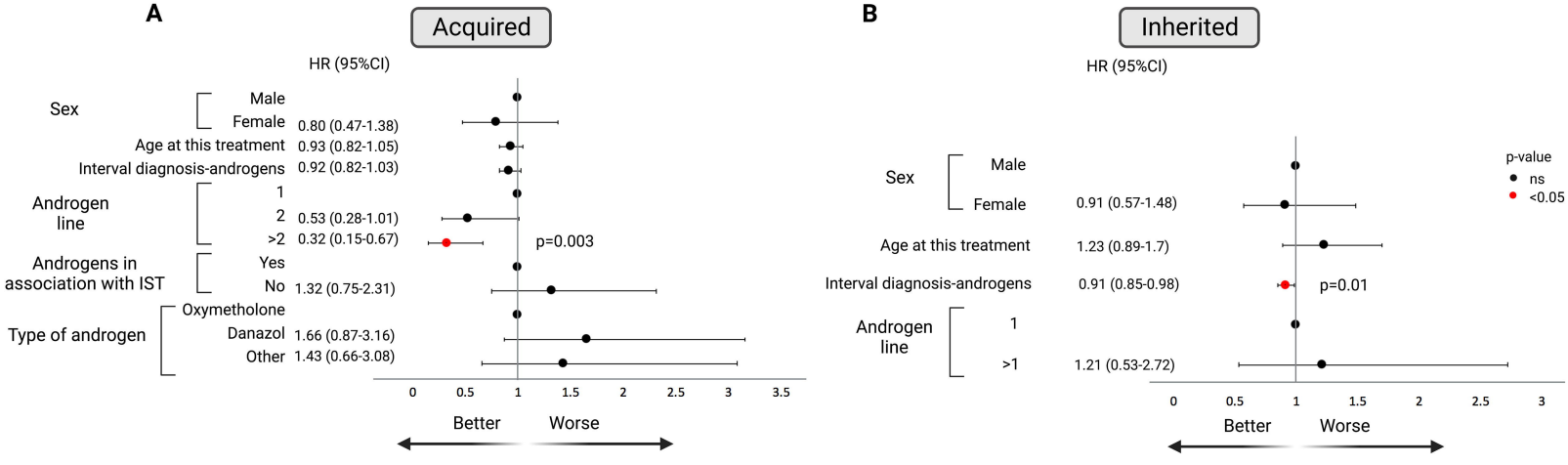

A
N=27
N=20
PR
29%
CR
8%
SD
63%
PR
45%
SD
55%
Response rate (%)
100
80
60
40
20
0
3 Months
6 Months
Response to androgen treatment
B
OS
95% (90-100%)
82% (74-91%)
78% (68-87%)
Survival (%)
100
75
50
25
0
0
12
24
36
48
60
Months since Androgens start
81
77
71
60
48
41
C
TFS
69% (59-79%)
39% (29-50%)
17% (9-26%)
Survival (%)
100
75
50
25
0
0
12
24
36
48
60
Months since Androgens start
81
56
45
31
23
13
D
FFS
67% (56-77%)
37% (27-48%)
14% (6-22%)
100
75
50
25
0
0
12
24
36
48
60
Months since Androgens start
81
54
42
30
23
11

Figure 4


A
Acquired
HR (95%CI)
Sex
Male
Female
0.80 (0.47-1.38)
Age at this treatment
0.93 (0.82-1.05)
Interval diagnosis-androgens
0.92 (0.82-1.03)
Androgen line
1
2
0.53 (0.28-1.01)
>2
0.32 (0.15-0.67)
p=0.003
Androgens in association with IST
Yes
No
1.32 (0.75-2.31)
Type of androgen
Oxymetholone
Danazol
1.66 (0.87-3.16)
Other
1.43 (0.66-3.08)
0
0.5
1
1.5
2
2.5
3
3.5
Better
Worse
B
Inherited
HR (95%CI)
Sex
Male
Female
0.91 (0.57-1.48)
Age at this treatment
1.23 (0.89-1.7)
Interval diagnosis-androgens
0.91 (0.85-0.98)
p=0.01
Androgen line
1
>1
1.21 (0.53-2.72)
p-value
ns
<0.05
0
0.5
1
1.5
2
2.5
3
Better
Worse

Supplementary Appendix

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

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Table of contents

Supplementary figures

Figure S1

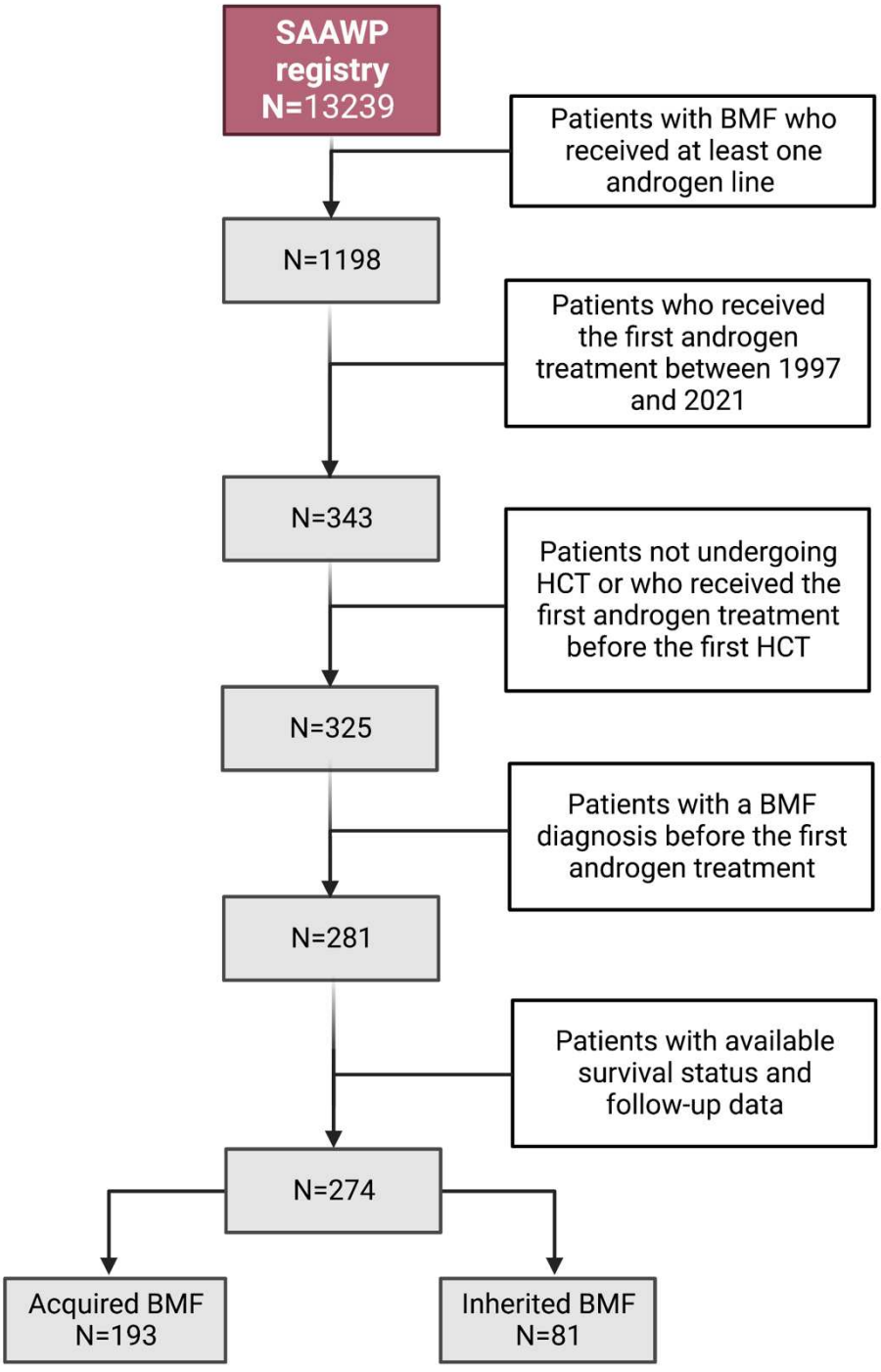

Figure S1: CONSORT diagram. Flow chart of selection criteria for identified androgen-treated patients in SAAWP registry. Abbreviations: BMF: Bone marrow failure; HCT: hematopoietic cell transplantation.

Figure S2

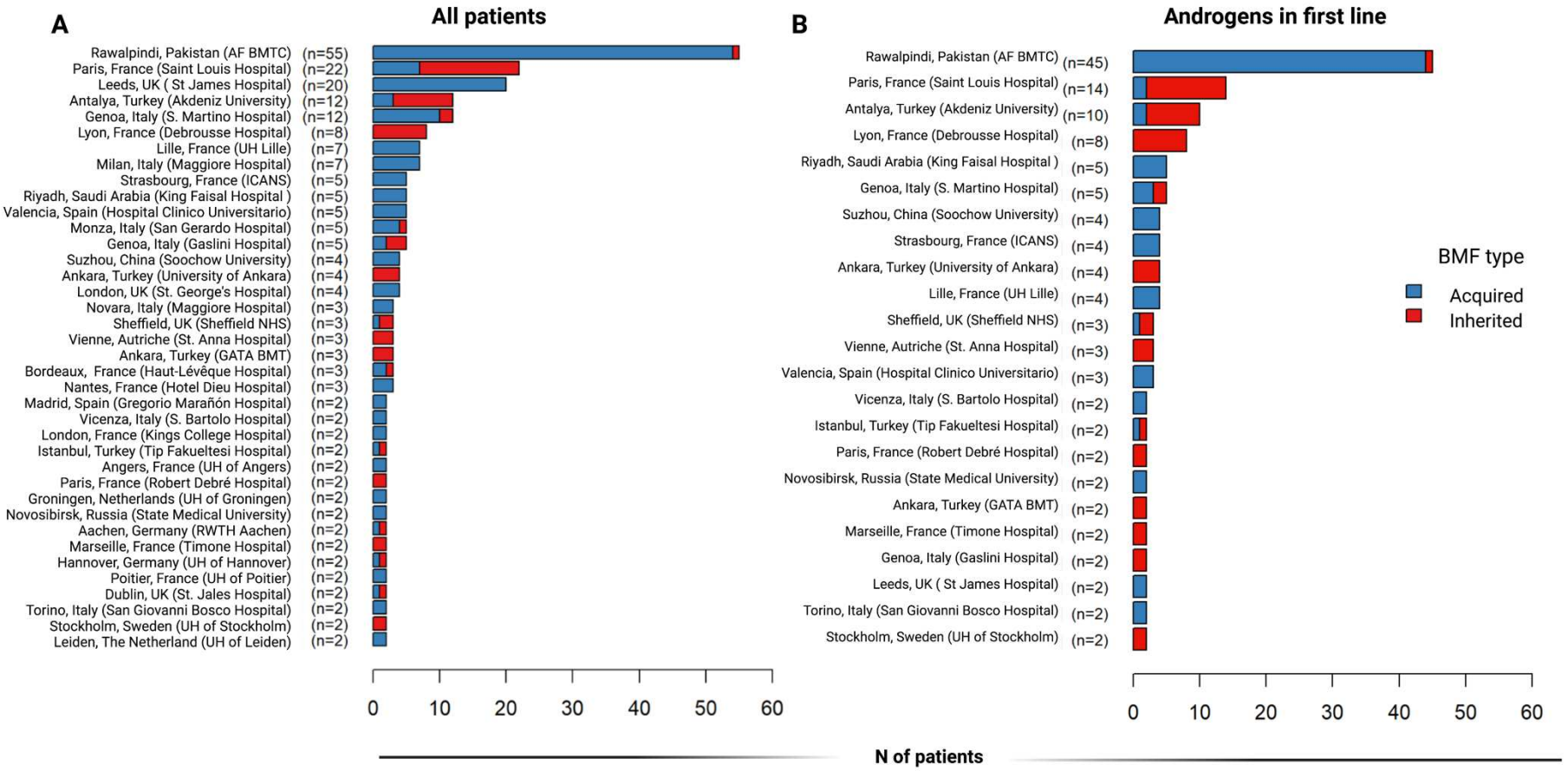
A
All patients
Rawalpindi, Pakistan (AF BMTC) (n=55)
Paris, France (Saint Louis Hospital) (n=22)
Leeds, UK (St James Hospital) (n=20)
Antalya, Turkey (Akdeniz University) (n=12)
Genoa, Italy (S. Martino Hospital) (n=12)
Lyon, France (Debrousse Hospital) (n=8)
Lille, France (UH Lille) (n=7)
Milan, Italy (Maggiore Hospital) (n=7)
Strasbourg, France (ICANS) (n=5)
Riyadh, Saudi Arabia (King Faisal Hospital) (n=5)
Valencia, Spain (Hospital Clinico Universitario) (n=5)
Monza, Italy (San Gerardo Hospital) (n=5)
Genoa, Italy (Gaslini Hospital) (n=5)
Suzhou, China (Soochow University) (n=4)
Ankara, Turkey (University of Ankara) (n=4)
London, UK (St. George's Hospital) (n=4)
Novara, Italy (Maggiore Hospital) (n=3)
Sheffield, UK (Sheffield NHS) (n=3)
Vienne, Autriche (St. Anna Hospital) (n=3)
Ankara, Turkey (GATA BMT) (n=3)
Bordeaux, France (Haut-Lévêque Hospital) (n=3)
Nantes, France (Hotel Dieu Hospital) (n=3)
Madrid, Spain (Gregorio Marañón Hospital) (n=2)
Vicenza, Italy (S. Bartolo Hospital) (n=2)
London, France (Kings College Hospital) (n=2)
Istanbul, Turkey (Tip Fakueltesi Hospital) (n=2)
Angers, France (UH of Angers) (n=2)
Paris, France (Robert Debré Hospital) (n=2)
Groningen, Netherlands (UH of Groningen) (n=2)
Novosibirsk, Russia (State Medical University) (n=2)
Aachen, Germany (RWTH Aachen) (n=2)
Marseille, France (Timone Hospital) (n=2)
Hannover, Germany (UH of Hannover) (n=2)
Poitier, France (UH of Poitier) (n=2)
Dublin, UK (St. Jales Hospital) (n=2)
Torino, Italy (San Giovanni Bosco Hospital) (n=2)
Stockholm, Sweden (UH of Stockholm) (n=2)
Leiden, The Netherland (UH of Leiden) (n=2)
0 10 20 30 40 50 60
B
Androgens in first line
Rawalpindi, Pakistan (AF BMTC) (n=45)
Paris, France (Saint Louis Hospital) (n=14)
Antalya, Turkey (Akdeniz University) (n=10)
Lyon, France (Debrousse Hospital) (n=8)
Riyadh, Saudi Arabia (King Faisal Hospital) (n=5)
Genoa, Italy (S. Martino Hospital) (n=5)
Suzhou, China (Soochow University) (n=4)
Strasbourg, France (ICANS) (n=4)
Ankara, Turkey (University of Ankara) (n=4)
Lille, France (UH Lille) (n=4)
Sheffield, UK (Sheffield NHS) (n=3)
Vienne, Autriche (St. Anna Hospital) (n=3)
Valencia, Spain (Hospital Clinico Universitario) (n=3)
Vicenza, Italy (S. Bartolo Hospital) (n=2)
Istanbul, Turkey (Tip Fakueltesi Hospital) (n=2)
Paris, France (Robert Debré Hospital) (n=2)
Novosibirsk, Russia (State Medical University) (n=2)
Ankara, Turkey (GATA BMT) (n=2)
Marseille, France (Timone Hospital) (n=2)
Genoa, Italy (Gaslini Hospital) (n=2)
Leeds, UK (St James Hospital) (n=2)
Torino, Italy (San Giovanni Bosco Hospital) (n=2)
Stockholm, Sweden (UH of Stockholm) (n=2)
0 10 20 30 40 50 60
BMF type
Acquired
Inherited
N of patients

Figure S3

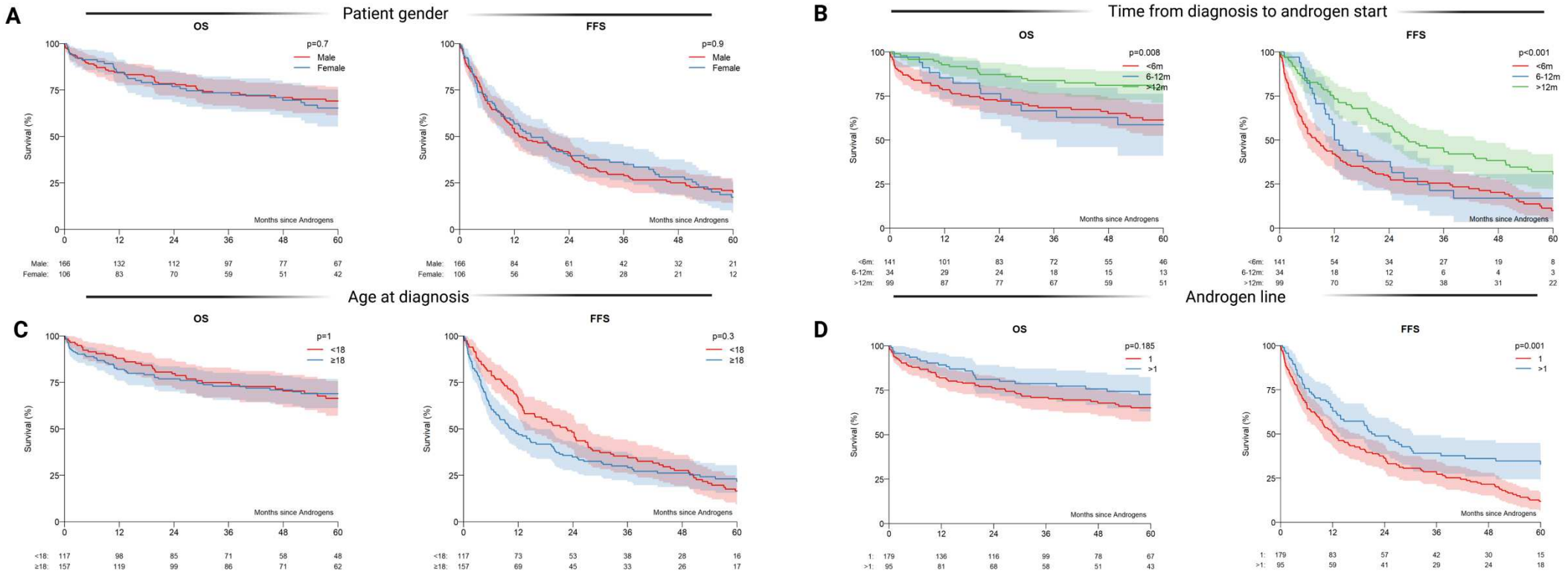

Figure S3: Univariable analysis of baseline variables impacting OS and FFS for acquired BMF. Kaplan-Meyer estimations of overall survival (OS) and failure free survival (FFS) of patients with acquired BMF showing the impact of **A)** Age, **B)** the time for androgen start, **C)** the age at diagnosis, **D)** the line of therapy (1: androgens given in first line, >1 androgen given after the first line).

Figure S4

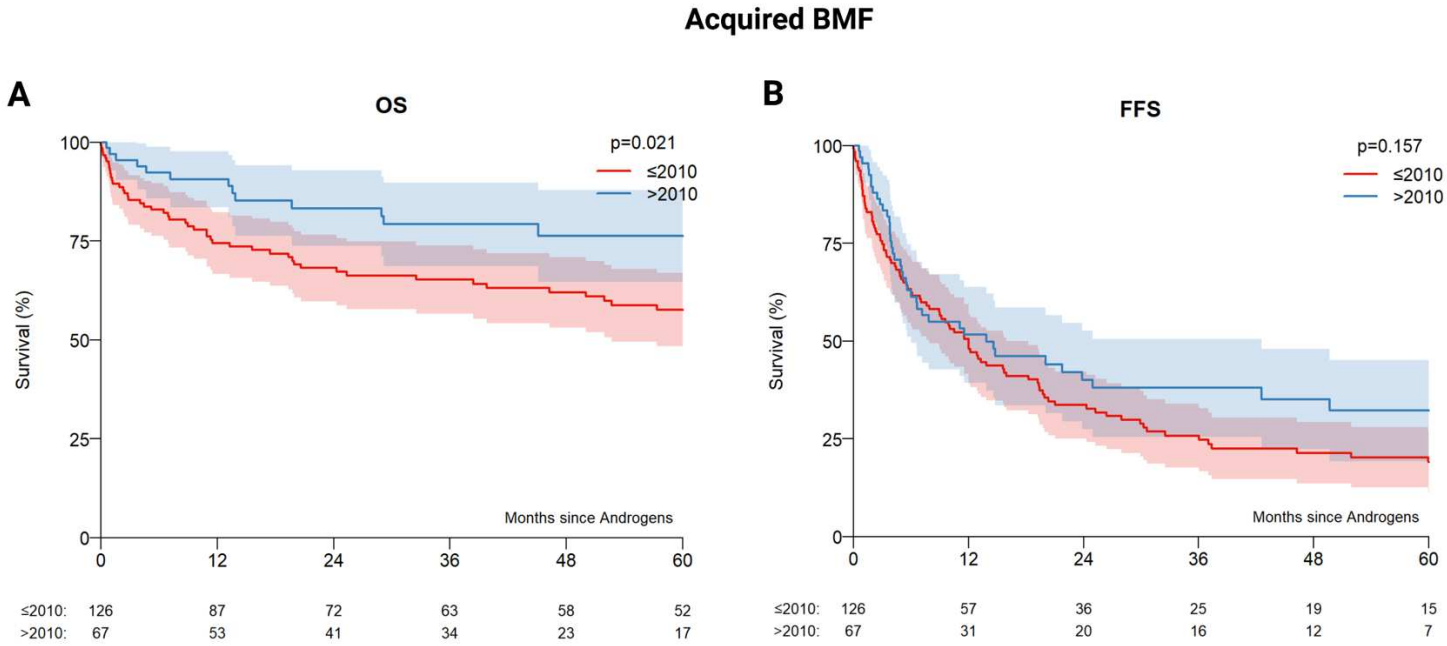

Figure S4: Univariable analysis of impact of year of treatment for acquired BMF. Kaplan-Meyer estimations of overall survival (A) and failure free survival (B) of patients with acquired BMF showing the impact of year of treatment.

Figure S5

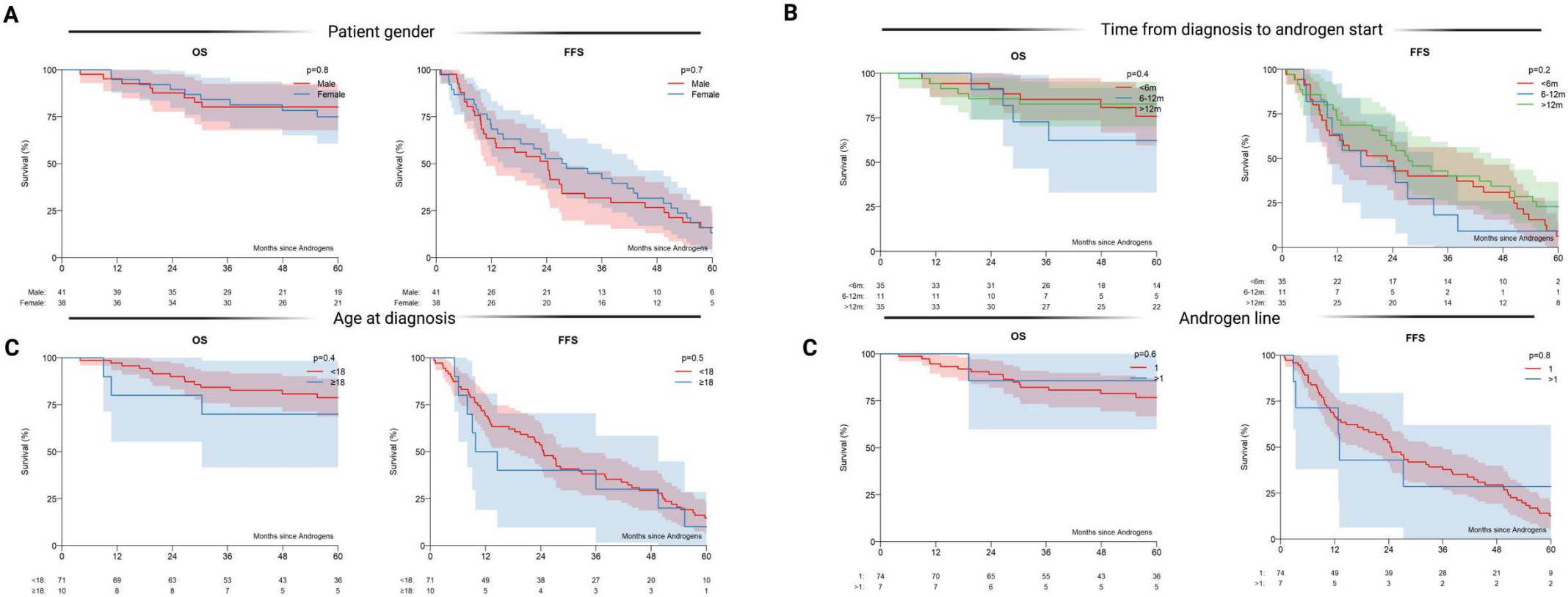

Figure S5: Figure S3: Univariable analysis of baseline variables impacting OS and FFS for inherited BMF. Kaplan-Meyer estimations of overall survival (OS) and failure free survival (FFS) of patients with inherited BMF showing the impact of **A)** Age, **B)** the time for androgen start, **C)** the age at diagnosis, **D)** the line of therapy (1: androgens given in first line, >1 androgen given after the first line).

Figure S6

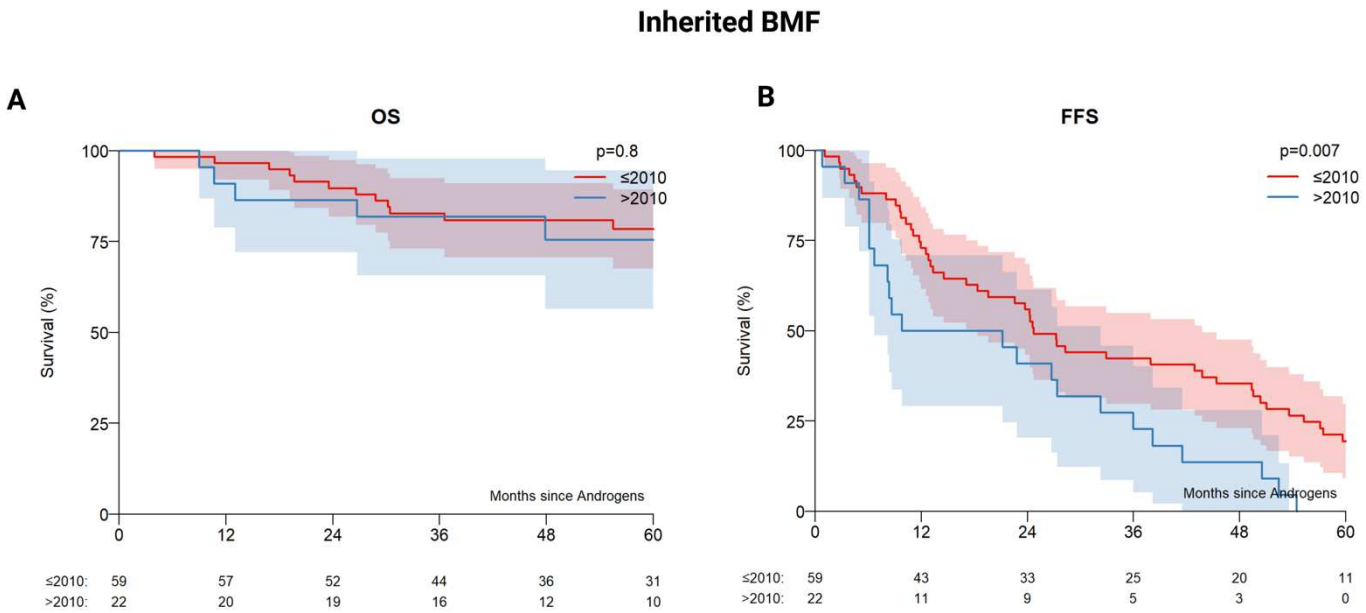

Figure S6: Univariable analysis of impact of year of treatment for inherited BMF. Kaplan-Meyer estimations of overall survival and failure free survival of patients with inherited BMF showing the impact of year of treatment.

Figure S7

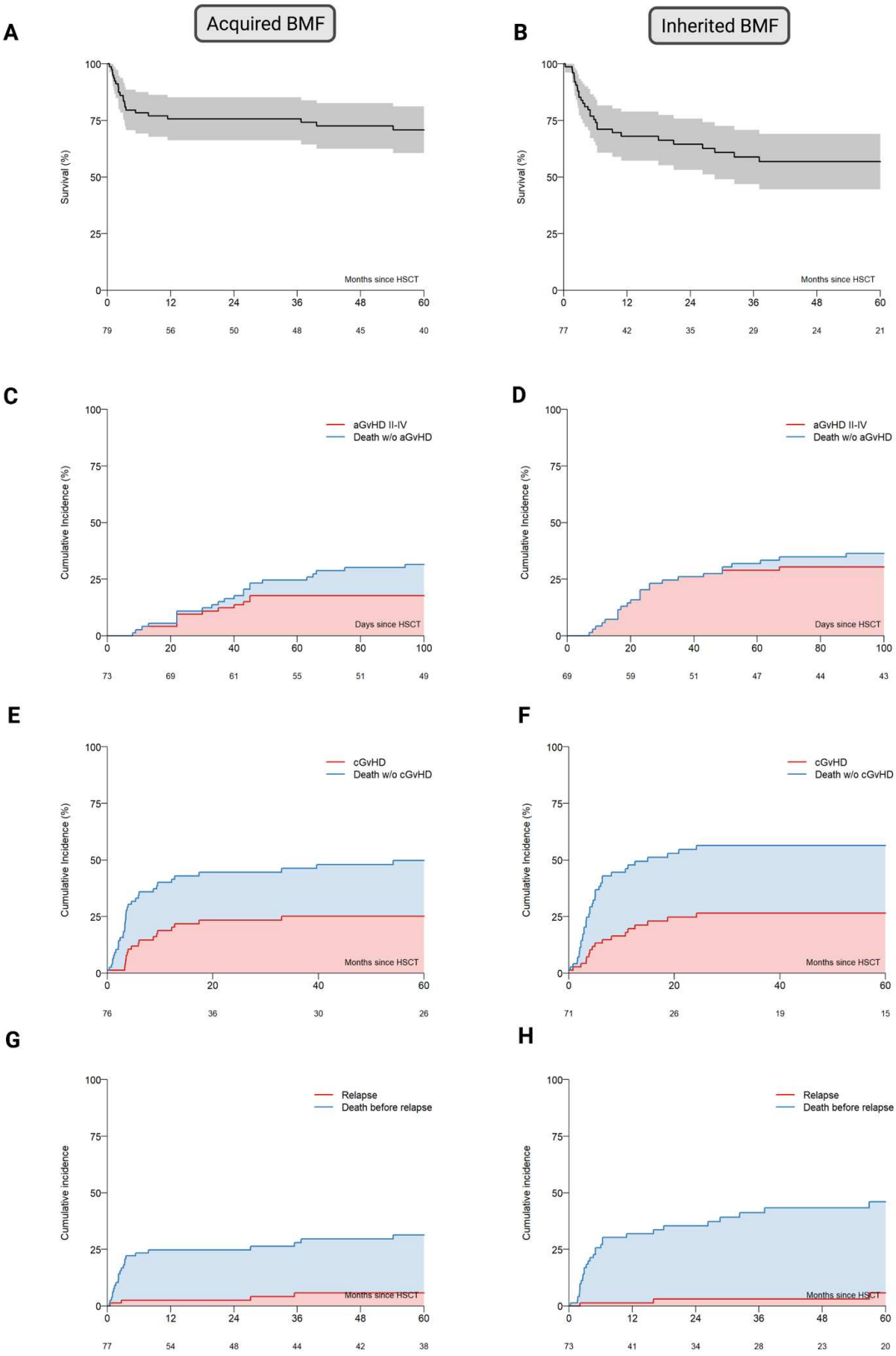
A
Acquired BMF
Survival (%)
Months since HSCT
79 56 50 48 45 40
B
Inherited BMF
Survival (%)
Months since HSCT
77 42 35 29 24 21
C
Cumulative Incidence (%)
aGvHD II-IV
Death w/o aGvHD
Days since HSCT
73 69 61 55 51 49
D
Cumulative Incidence (%)
aGvHD II-IV
Death w/o aGvHD
Days since HSCT
69 59 51 47 44 43
E
Cumulative Incidence (%)
cGvHD
Death w/o cGvHD
Months since HSCT
76 36 30 26
F
Cumulative Incidence (%)
cGvHD
Death w/o cGvHD
Months since HSCT
71 26 19 15
G
Cumulative incidence
Relapse
Death before relapse
Months since HSCT
77 54 48 44 42 38
H
Cumulative incidence
Relapse
Death before relapse
Months since HSCT
73 41 34 28 23 20

Figure S7: Outcome analysis in patients receiving an allogeneic hematopoietic cell transplantation after androgen treatment. A) Kaplan-Meyer estimates of OS in acquired BMF patients. B) Kaplan-Meyer estimates of OS in inherited BMF patients. C) Cumulative incidence of acute GvHD in acquired BMF patients (red); competing events are shown in blue. D) Cumulative incidence of acute GvHD in inherited BMF patients (red); competing events are shown in blue. E) Cumulative incidence of chronic GvHD in acquired BMF patients (red); competing events are shown in blue. F) Cumulative incidence of acute GvHD in inherited BMF patients (red); competing events are shown in blue. G) Cumulative incidence of relapse in acquired BMF patients (red); competing events are shown in blue; H) Cumulative incidence of acute GvHD in inherited BMF patients (red); competing events are shown in blue.

Supplementary tables

Table S1

Table S1: Patient characteristics in acquired BMF group according to androgen-line					
Variable		**1st line**		**>1st line**	
		N (%)/Median(IQR)	Missing	N (%)/Median(IQR)	Missing
All		105 (54.4%)		88 (45.6%)	
Type of diagnosis	Aplastic anemia	94 (89.5%)		82 (93.2%)	
	Pure red cell aplasia	4 (3.8%)		2 (2.3%)	
	Paroxysmal nocturnal hemoglobinuria (PNH)	5 (4.8%)		1 (1.1%)	
	Amegakaryocytic thrombocytopenia	1 (1.0%)		2 (2.3%)	
	Other acquired cytopenic syndromes	1 (1.0%)		1 (1.1%)	
Severity of Aplastic anemia	Moderate	15 (20.0%)	30 (28.6%)	24 (32.9%)	15 (17.0%)
	Severe	43 (57.3%)		31 (42.5%)	
	Very severe	17 (22.7%)		18 (24.7%)	
Sex	Male	73 (69.5%)		52 (59.1%)	
	Female	32 (30.5%)		36 (40.9%)	
Age at this diagnosis (years)		26.2 (16.8-43.2)		39.6 (18.9-57.9)	
Hemoglobin (g/dl) at diagnosis		7.8 (5.8-9.6)	31 (29.5%)	8 (6.3-9.4)	19 (21.6%)
Neutrophils (x10ˆ9/L) at diagnosis		0.8 (0.4-1.2)	30 (28.6%)	0.7 (0.2-1.2)	21 (23.9%)
Platelets (x10^9/L) at diagnosis		8.5 (5-20)	29 (27.6%)	14 (8-25.2)	20 (22.7%)
Age at treatment initiation (years)		27 (17.3-43.2)		41.2 (22.1-60.1)	
Interval diagnosis-androgen treatment (months)		0.4 (0-2.1)		17.6 (8.2-28.5)	

Reticulocytes on first androgen treatment		30.5 (11.8-55.5)	97 (92.4%)	43 (20-60)	59 (67.0%)
Neutrophils on first androgen treatment		0.8 (0.4-1)	71 (67.6%)	1.2 (0.9-1.9)	40 (45.5%)
Platelets on first androgen treatment		7.5 (3.8-19.2)	69 (65.7%)	22 (15-39)	39 (44.3%)
Transfused on first androgen treatment (RBC)	No	9 (29%)	74 (70.5%)	3 (6%)	38 (43.2%)
	Yes	22 (71%)		47 (94%)	
Transfused on first androgen treatment (Platelets)	No	8 (28.6%)	77 (73.3%)	5 (10.2%)	39 (44.3%)
	Yes	20 (71.4%)		44 (89.8%)	
Number of RBC transfusions on first androgen treatment	<20 units	44 (71.0%)	43 (41.0%)	13 (33.3%)	49 (55.7%)
	20-50 units	8 (12.9%)		15 (38.5%)	
	>50 units	4 (6.5%)		6 (15.4%)	
	None	6 (9.7%)		5 (12.8%)	
Number of platelet transfusions on first androgen treatment	<20 units	42 (68.9%)	44 (41.9%)	17 (43.6%)	49 (55.7%)
	20-50 units	7 (11.5%)		11 (28.2%)	
	>50 units	4 (6.6%)		4 (10.3%)	
	None	8 (13.1%)		7 (17.9%)	
Number of lines before androgens	0	105 (100.0%)		0 (0.0%)	
	1	0 (0.0%)		44 (50.0%)	
	2	0 (0.0%)		25 (28.4%)	
	>2	0 (0.0%)		19 (21.6%)	
Type of Androgen	Danazol	3 (5.7%)	52 (49.5%)	25 (42.4%)	29 (33.0%)
	Nandrolone	0 (0.0%)		1 (1.7%)	
	Oxymetholone	45 (84.9%)		19 (32.2%)	
	Other	0 (0.0%)		2 (3.4%)	
	Nilevar	3 (5.7%)		2 (3.4%)	
	Danatrol	1 (1.9%)		5 (8.5%)	
	Testosterone	1 (1.9%)		5 (8.5%)	

Duration first androgen treatment (months)	Median (IQR)	4 (1.7-17.6)	53 (50.5%)	8.8 (3.4-25.2)	37 (42.0%)

Abbreviations: BMF: bone marrow failure, IQR: interquartile range, RBC: red blood cell

Table S2

	Table S2: Univariable stratified analysis										
	Variable	Group	N	OS (95%CI lower-upper)				EFS (95%CI lower-upper)			
				12 months	36 months	60 months	p-value	12 months	36 months	60 months	p-value
Acquired BMF	**Sex**	Male	125	81% (74-88%)	71% (63-80%)	65% (56-75%)	0.5	50% (41-59%)	29% (21-38%)	22% (14-30%)	>0.99
		Female	68	78% (68-88%)	67% (56-79%)	60% (46-73%)		50% (38-62%)	32% (20-44%)	25% (13-38%)	
	Age	<18	46	73% (60-86%)	60% (45-75%)	45% (28-62%)	**0.016**	60% (45-74%)	31% (17-46%)	22% (9-36%)	0.7
		18	147	82% (76-89%)	73% (66-81%)	69% (61-77%)		47% (38-55%)	29% (21-37%)	23% (15-31%)	
	Months from diagnosis to androgen start	<12m	129	74% (66-82%)	63% (54-72%)	56% (47-66%)	**0.003**	37% (29-46%)	21% (13-28%)	16% (9-24%)	**<0.001**
		>12m	64	92% (85-99%)	84% (75-94%)	77% (66-89%)		74% (64-85%)	47% (34-60%)	36% (23-50%)	
	Androgen line	1	105	73% (64-81%)	63% (53-73%)	56% (45-67%)	0.06	38% (28-48%)	21% (12-29%)	14% (6-22%)	**<0.001**
		2	44	83% (72-95%)	78% (66-91%)	73% (59-87%)		51% (36-66%)	31% (17-45%)	19% (4-33%)	
		>2	44	93% (86-100%)	78% (65-91%)	70% (54-85%)		75% (62-88%)	49% (34-65%)	46% (30-62%)	
	Year of androgen treatment	2010	126	74% (67-82%)	65% (57-74%)	58% (48-67%)	**0.021**	49% (40-58%)	26% (18-34%)	19% (11-27%)	0.16
		>2010	67	91% (84-98%)	79% (69-90%)	76% (65-88%)		52% (39-64%)	38% (26-51%)	32% (19-45%)	
Inherited BMF	**Sex**	Male	41	95% (89-100%)	80% (68-92%)	80% (68-92%)	0.8	63% (49-78%)	32% (17-46%)	16% (4-27%)	0.7
		Female	38	95% (88-100%)	84% (72-96%)	75% (61-89%)		68% (54-83%)	42% (26-58%)	13% (2-24%)	
	Age	<18	71	97% (93-100%)	84% (76-93%)	79% (69-89%)	0.4	69% (58-80%)	38% (27-49%)	15% (6-23%)	0.5
		18	10	80% (55-100%)	70% (42-98%)	70% (42-98%)		50% (19-81%)	30% (2-58%)	10% (0-29%)	
	Months from diagnosis to androgen start	<12m	46	96% (90-100%)	82% (71-93%)	73% (58-87%)	0.5	63% (49-77%)	35% (21-49%)	7% (0-15%)	0.11
		>12m	35	94% (87-100%)	83% (70-95%)	83% (70-95%)		71% (56-86%)	40% (24-56%)	23% (9-37%)	
	Androgen line	1	74	95% (89-100%)	82% (73-91%)	77% (67-87%)	0.6	66% (55-77%)	38% (27-49%)	13% (5-20%)	0.8
		>1	7	100% (0-100%)	86% (60-100%)	86% (60-100%)		71% (38-100%)	29% (0-62%)	29% (0-62%)	
	Year of androgen treatment	2010	59	97% (92-100%)	83% (73-92%)	78% (67-89%)	0.8	73% (62-84%)	42% (30-55%)	19% (9-30%)	**0.007**
		>2010	22	91% (79-100%)	82% (66-98%)	76% (57-95%)		50% (29-71%)	23% (5-40%)		

Abbreviations: BMF: bone marrow failure, IQR: interquartile range, N: number; CI: confident interval, OS: Overall survival, FFS: Failure free survival.

Table S3

	Table S3: Cumulative incidence of toxicity						
	Type of toxicity	**N**	**N events**	**Median time to toxicity (months) (IQR)**	**Cumulative incidence of toxicity (95% CI)**		
					12 months	**36 months**	**60 months**
Acquired BMF	Liver toxicity	110	13	2.8 (0.5 - 5.7)	11% (5-18%)	13% (6-19%)	13% (6-19%)
	Gastrointestinal toxicity	109	4	6.3 (4.5 - 7.3)	4% (0-8%)	4% (0-8%)	4% (0-8%)
	Psychiatric toxicity	111	1	18.1 (18.1 - 18.1)	0% (0-0%)	1% (0-3%)	1% (0-3%)
	Renal toxicity	109	3	5.4 (4.4 - 5.8)	3% (0-6%)	3% (0-6%)	3% (0-6%)
	Endocrinological toxicity	0					
Inherited BMF	Liver toxicity	31	4	22.8 (8 - 41.5)	6% (0-15%)	10% (0-20%)	13% (1-25%)
	Gastrointestinal toxicity		0				
	Psychiatric toxicity		0				
	Renal toxicity		0				
	Endocrinological toxicity	31	2	15.1 (11.5 - 18.7)	3% (0-9%)	6% (0-15%)	6% (0-15%)

Abbreviations: BMF: bone marrow failure, IQR: interquartile range, N: number; CI: confident interval

Table S4

Table S4: Patient characteristics of the transplanted cohort					
		Acquired		Inherited	
	Group	N (%)/median(IQR)	Missing	N (%)/median(IQR)	Missing
		N=82		N=70	
Age at this treatment	Median (IQR)	30.1 (22-42.5)		11.6 (7.9-15.5)	
Interval diagnosis-tx in months	Median (IQR)	19.7 (9.6-55.6)		41 (16.7-70.3)	
Stem cell source	BM	45 (58%)	4 (4.9%)	37 (48.1%)	2 (4.5%)
	PB	24 (31%)		25 (32.5%)	
	CB	4 (5%)		1 (1.3%)	
	Mixed graft	5 (6%)		14 (18.20%)	
Type of donor	Identical sibling	24 (30.4%)	3 (3.7%)	21 (27.30%)	1 (2.3%)
	Matched other relative			5 (6.50%)	
	Matched unrelated	11 (13.9%)		11 (14.30%)	
	Mismatched relative	10 (12.7%)		9 (11.70%)	
	Mismatched unrelated	10 (12.7%)		12 (15.60%)	
	Unrelated	24 (30.4%)		19 (24.70%)	
Interval start first androgen treatment to first tx (months)	Median (IQR)	14.3 (4.9-32.4)		25.1 (9.9-50.5)	
GvHD Prophylaxis	CNI/MTX	47 (62%)	6 (7%)	12 (17%)	8 (10%)
	CNI alone	13 (17%)		33 (47%)	
	CNI/MMF	11 (14%)		23(33%)	
	Other	5 (7%)		2 (3%)	
Conditioning regimen	RIC	43 (56.6%)	6 (7.3%)	55 (76.4%)	6 (7.7%)
	MAC	33 (43.4%)		17 (23.6%)	

Abbreviations: BMF: bone marrow failure; IQR: interquartile range, N: number; tx: transplant; GvHD: graft versus host disease; CNI: calcineurin inhibitor; MTX: methotrexate ; MMF : Mycophenolate mofetil ; RIC : reduced intensity conditioning ; MAC : myeloablative conditioning regimen ; NM : non myeloablative