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GVHD Prophylaxis and Incidence in Contemporary AlloHSCT: A Large Real-World GETH/TC Analysis.

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Table 1 to 3 are available in the Supplementary Files section.

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GVHD Prophylaxis and Incidence in Contemporary AlloHSCT: A Large Real-World GETH/TC Analysis

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ABSTRACT

Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (alloHSCT). Although post-transplant cyclophosphamide (PTCy) and antithymocyte globulin (ATG) have reshaped prophylactic strategies, contemporary real-world data on GVHD incidence under current practice are limited. We conducted a nationwide retrospective study including 1 544 alloHSCTs performed in Spain (2018-2020) to characterize current prophylaxis patterns and the epidemiology of GVHD. PTCy-based prophylaxis was used in 65.6% of transplants, being nearly universal in haploidentical donors (96.9%) and predominant in mismatched unrelated (72.4%), matched unrelated (58.6%), and matched related donors (43.5%). The cumulative incidence of acute GVHD grades II-IV was 28.4% at day +100 and 30.7% at 1 year; grade III-IV occurred in 6.9% and 8.0%, respectively. Chronic GVHD at 2 years was 36.7%, with 23.5% moderate-to-severe cases. Acute GVHD was independently associated with inferior survival (HR 2.40, $p<0.001$), particularly grade III-IV disease (HR 4.34, $p<0.001$). These findings define the contemporary real-world burden of GVHD in a high-volume European country and provide an updated epidemiologic framework to inform preventive strategies, clinical trial design, and healthcare planning in modern alloHSCT.

INTRODUCTION

Graft-versus-host disease (GVHD) is a systemic disorder that occurs when donor immune cells recognize the host as foreign and attack recipient tissues after allogeneic hematopoietic stem cell transplantation (alloHSCT).[1] Acute and chronic GVHD are associated with morbidity, mortality, impaired quality of life, prolonged immunosuppressive therapy, and increased infection risk after transplant. Therefore, improving prevention and treatment of GVHD remains a central objective of clinical research.

Historically, with standard GVHD prophylaxis combining a calcineurin inhibitor (CNI) (tacrolimus or cyclosporine) with methotrexate (MTX) or mycophenolate mofetil (MMF), the incidence of acute and chronic GVHD has been estimated at 30-50% and 50-70%, respectively.[2–7] Over the past decade, in vivo T-cell depletion strategies using antithymocyte globulin (ATG) and high-dose post-transplant cyclophosphamide (PTCy) have increasingly been adopted and have reduced GVHD incidence.[8–18] However, these regimens are not universally implemented. Variability in clinical practice across and within countries may significantly influence the actual incidence of GVHD.

To evaluate the current burden of GVHD and justify resource allocation for preclinical and clinical research, it is essential to reassess its contemporary incidence. Key considerations include evolving preventive and therapeutic approaches, biomarker development, personalized strategies, and implementation challenges. In the United States (US), mean total all-cause costs within the first 100 days post-transplant are approximately $316 458 for patients with acute GVHD versus $215 229 without GVHD; annual costs for chronic GVHD exceed $300 000 per patient.[19]

Real-world studies provide valuable insights. Spain is among the most active European countries in alloHSCT, with 200-300 procedures per 10 million population in 2022.[20] The number has steadily increased, reaching 1 478 in 2 023. We hypothesized that contemporary GVHD incidence in Spain differs from historical series due to changes in donor selection and prophylaxis. Accordingly, we aimed to describe current GVHD prophylaxis practices in Spain, determine the cumulative incidence of acute and chronic GVHD, and assess its impact on post-transplant survival.

METHODS

Study design

This retrospective study analyzed alloHSCT procedures performed in 13 Spanish centers, selected for their high activity and for reporting at least 95% of their cases during the period of study through the European Bone Marrow Transplant (EBMT) and *Grupo Español del Trasplante hematopoyético y Terapia Celular* (GETH-TC) registries.

First and second alloHSCT procedures performed in patients ≥18 years between January 2018 and December 2020 were included. AlloHSCT from HLA-identical related (MRD) and unrelated donors (MUD), HLA-mismatched related (MMRD) and unrelated (MMUD) as well as from haploidentical donors, were included. AlloHSCT from umbilical cord blood were excluded from the study due to their low prevalence during the study period (<1%).

Main recorded variables were GVHD prophylaxis regimen and acute and chronic GVHD together with age, sex, hematopoietic comorbidity risk index (HCT-CI), Karnofsky performance score, underlying disease, disease status at the time of alloHSCT, disease risk index, date of stem cell infusion, conditioning regimen, type of donor, source of stem cells, use of manipulated graft and type, and serological CMV status.

Study data were collected retrospectively and managed using Research Electronic Data Capture (REDCap. Copyright 2006-2013 Vanderbilt University; all rights reserved) hosted by GETH-TC. The study was approved by the Ethics Committee of Hospital Clínico Universitario of Valencia on June 16th 2023 (code number 400/2023), and adhered to the ethical standards of the Declaration of Helsinki. At the time of the alloHSCT, the patient signed an informed consent form requesting authorization for

the registration of his or her data in the EBMT Registry and authorization for the transfer of these data to the Spanish Registry GETH-TC.

GVHD diagnosis and staging

The GETH-TC Spanish centers uniformly use the currently recommended criteria for the diagnosis and staging of GVHD.[21-23] Therefore, the acute GVHD was graded in accordance with the MAGIC criteria, while chronic GVHD was diagnosed and graded based on the 2014 NIH Consensus Criteria.[21-23] All patients were managed upfront with curative intent strategies, and following standard practices and protocols from each institution.

Study endpoints and statistical analysis

The objectives of this study were (1) to describe the current GVHD prophylaxis regimens used in Spain, (2) to evaluate the cumulative incidence of acute and chronic GVHD, and (3) to assess the impact of GVHD on post-transplant survival. GVHD prophylaxis was considered the main explanatory variable. Data was analyzed globally and according to donor type, as this variable showed a strong correlation with the GVHD prevention strategy applied.

Descriptive statistics were employed to summarize categorical and continuous variables, reporting counts, percentages, medians, and ranges. Time-to-event analyses were conducted from the date of transplant to the date of event or last follow-up. Overall survival (OS), disease-free survival (DFS), and GVHD-Relapse-free survival (GRFS) were estimated using the Kaplan–Meier method, with GRFS accounting for death, relapse, grades III and IV acute GVHD, and moderate-to-severe chronic GVHD as

events. Cumulative incidences of GVHD, NRM, and relapse were estimated using Fine and Gray's competing risk analysis, considering death and relapse as competing events for GVHD, relapse as a competing event for NRM, and death without relapse as competing event for CIR.

Statistical significance was defined at the 0.05 level. Analysis was performed using StataCorp. 2025 (Stata Statistical Software: Release 19. College Station, TX: StataCorp LLC).

RESULTS

Characteristics of the patients and GVHD prophylaxis

A total of 1 544 alloHSCT procedures were registered in the database. During the study period, four main GVHD prophylaxis strategies were employed **(Table 1)**. The combination of a CNI plus MTX was used in 203 patients (13.1%), while CNI plus sirolimus accounted for 117 cases (7.6%). ATG-based prophylaxis (ATG plus CNI and MTX or MMF) was employed in 118 patients (7.6%). PTCy-based prophylaxis (PTCy alone, PTCy plus CNI with/without MMF, and PTCy plus sirolimus and MMF) progressively became the dominant strategy, administered in 1 013 transplants (65.6%). In 93 cases, other immunosuppressant combinations were used, and they were not included in the analyses of GVHD incidence or post-transplant outcomes due to their high heterogeneity. A progressive increase in the use of PTCy-based prophylaxis was observed from 61% in 2018 to 70% in 2020, whereas CNI-MTX and CNI-Sirolimus decreased from 15% to 11% and 10% to 7%, respectively **(Supplementary Figure 1)**.

Main characteristics of patients and alloHSCT procedure are shown in **Supplementary Table 1.** The median age of the study cohort was 54 years, and 56.0% of patients were male. The most frequent diagnoses were acute myeloid leukemia (35.8%), myelodysplastic syndrome (15.0%), non-Hodgkin lymphoma (13.1%), and acute lymphoblastic leukemia (11.9%). Conditioning regimens were myeloablative (MAC) in 41.2% of the cases and non-myeloablative (NMA) or reduced intensity conditioning (RIC) regimens in 58.8%. Donor types included MRD (32.9%), MUD (29.6%), haploidentical (27.7%), MMUD (8.7%), and MMRD (1.1%), and the majority of alloHSCT were performed using peripheral blood stem cell grafts (89.2%).

GVHD Prophylaxis by donor type

As expected, GVHD prophylaxis was strongly influenced by donor type, reflecting standard clinical practice and the need to tailor prophylaxis according to the degree of HLA disparity **(Table 1)**. The correlation between GVHD prophylaxis and donor type is illustrated in **Figure 1.** The use of PTCy-based prophylaxis was almost universal in haploidentical transplants (96.9%) and highly prevalent in MMRD/MMUD settings (72.4%). In contrast, a higher variability was observed in MSD and MUD alloHSCT, where PTCy was less frequent but still represented 43.5% and 58.6% of cases, respectively. In addition, while PTCy-based, ATG-based, and CNI-MTX regimens were equally used with both MAC and RIC/NMA conditioning, CNI-Sirolimus was almost exclusively restricted to the RIC/NMA settings **(Table 1).**

Cumulative incidences of acute GVHD

Given the heterogeneity of prophylactic regimens in the "Other" cohort, the cumulative incidences of acute and chronic GVHD were evaluated only among the 1,451 patients who received one of the four main strategies applied across participating institutions. In addition, analyses were performed both in the overall population and separately by donor type, due to the strong association between these two variables in clinical practice.

The global incidence of grade II–IV acute GVHD was 28.4% (95% CI, 26.2–30.8) at day +100, and 30.7% (95% CI, 28.3–33.1) at 1 year (**Figure 2A**). By donor type, the corresponding incidences at day +100 were 28.1% (95% CI, 24.1–32.3) for MSD, 28.5% (95% CI 24.3-32.8) for MUD, 26.2% (95% CI, 19.4–33.5) for MMUD/MMRD, and 29.5%

(95% CI, 25.2–33.9) for haploidentical transplants **(Supplementary Figure 2 A-D)**. At 1 year, figures were 31.0% (95% CI, 26.8–35.3), 30.1% (95% CI, 25.9–34.5), 28.9% (95% CI, 21.9–36.4), and 31.4% (95% CI, 27.0–35.9), respectively.

Severe forms of acute GVHD (grades III-IV) were observed in 6.9% (95% CI, 5.7-8.3) at day +100 and 8.0% (95% CI, 6.7-9.5) at 1 year in the overall cohort **(Figure 2B).** By donor type, the corresponding incidences were: MSD 6.8% (95% CI 4.7-9.4) and 8.1% (95% CI 5.8-10.9), MUD 7.0% (95% CI 4.8-9.6) and 8.1% (95% CI 5.8-11.0), MMUD/MMRD 8.1% (95% CI 4.4-13.1) and 9.4% (95% CI 5.4-14.8), and haploidentical, 6.5% (95% CI 4.4-9.2) and 7.3% (95% CI 5.0-10.0), respectively **(Supplementary Figure 2 E-H).**

In comparison to other GVHD prophylaxis strategies, PTCy-based was significantly associated with lower risk of grade II-IV and grade III-IV acute GVHD in the global series of patients (**Table 2, Figure 3**).

Cumulative incidences of chronic GVHD

The cumulative incidence of global and moderate-severe chronic GVHD at 2-year post-transplant were 36.7% (95% CI 34.0-39.5) and 23.5% (95% CI 21.2-26.0) for the entire cohort (**Figure 4**). According to donor type, 2-year cumulative incidences of global chronic GVHD were 42.2% (95% CI 37.4-47.0) for MRD, 36.8% (95% CI 31.9-41.8) for MUD, 31.9% (95% CI 23.6-40.6) for MMUD/MMRD, and 31.5% (95% CI 26.5-36.6) for haploidentical **(Supplementary Figure 3).** The corresponding figures for moderate-severe chronic GVHD were 30.7% (95% CI 26.3-35.2) for MRD, 22.0% (95% CI 17.9-26.4) for MUD, 23.7% (95% CI 16.3-31.8) for MMUD/MMRD, and 16.3% (95% CI 12.5-20.5) for haploidentical.

The use of PTCy-prophylaxis was significantly associated with a lower incidence of global chronic GVHD and moderate-severe chronic GVHD in comparison to CNI-MTX and CNI-sirolimus (**Table 2, Figure 5**). No differences were observed between PTCy-based and ATG-based prophylaxis.

NRM, relapse, and survival after transplantation

After a median follow-up of 48 months (IQR, 39.5-57.5), the estimated 2-year OS for the entire cohort was 66.5% (95% CI 64.0-68.9). OS according to donor type was 70.2% for MRD, 69.6% for MUD, 60.5% for MMUD/MMRD, and 61.4% for haploidentical donors. The corresponding 2-year DFS rates were 59.2% (95% CI 56.6-61.8) for the whole cohort, and 60.2%, 61.9%, 56.4%, and 56.2% for MRD, MUD, MMUD/MMRD, and haploidentical donors, respectively. No significant differences in OS or DFS were observed according to the type of GVHD prophylaxis **(Supplementary Table 2)**.

The cumulative incidence of NRM for the entire cohort was 19.9% (95% CI 17.9-22.1) at 2 years. Patients who received ATG-based GVHD prophylaxis had the highest mortality (28.2%), whereas those who received CNI-Sirolimus prophylaxis had the lowest (10.3%). According to donor type, NRM was 14.5% for MRD, 17.0% for MUD, 25.8% for MMUD/MMRD, and 27.1% for haploidentical donors. The estimated cumulative incidence of relapse at 2 years was 20.8% (95% CI 18.8-23.0), ranging from 16.7% with haploidentical donors to 25.3% in MRD. The group of CNI-Sirolimus prophylaxis, constituted mainly by patients receiving RIC/NMA regimes, presented a significant higher risk of relapse (32.6%, HR 1.62, P=0.006) **(Supplementary Table 2)**.

The development of acute GVHD grades II-IV was significantly associated with poorer survival (HR 2.3, 95% CI 1.8-3.0, P<0.001), as illustrated in **Table 3**. This association remained consistent across different donor types **(Supplementary Figure 3)**. The impact was particularly notable in the case of GVHD grades III-IV (HR 4.2, 95% CI 2.8-6.3, P<0.001). There were no statistically significant differences in OS between patients who developed global or moderate-severe chronic GVHD and those who did not **(Supplementary Figure 4).**

DISCUSSION

Our study provides real-world evidence on current trends in GVHD prophylaxis across 13 institutions in Spain and describes the observed incidences of GVHD in routine clinical practice across all donor types. Importantly, the primary objective of this study was *descriptive*: to characterize current prophylactic strategies used nationwide and to document the real incidence of both acute and chronic GVHD in contemporary practice. The study was not designed nor powered to perform multivariable analyses of GVHD risk factors, nor to compare the effectiveness of different prophylaxis regimens. Therefore, any differences observed between prophylactic approaches should be interpreted strictly within this descriptive framework.

The most notable finding is the widespread adoption of PTCy, which has become almost universal in haploidentical transplantation, the preferred approach in mismatched donor transplants, and is increasingly used in both MRD and MUD settings. This shift mirrors trends reported in international registries.[24] Importantly, the broad use of PTCy translated into a reduction of both acute and chronic GVHD, particularly in severe forms, compared with historical series based on CNI-MTX/MMF. Moreover, our observations on the negative impact of acute GVHD on survival reinforce the notion that preventing GVHD must remain a primary objective of research in alloHSCT.

Historically, acute GVHD incidence after alloHSCT ranged from 40%-67% for grades II-IV and approximately 15% for grades III-IV.[2,5] An analysis from the EBMT

registry including 102,557 MRD or MUD transplants over five time periods (1990–2015) showed a progressive decline in 100-day acute GVHD: grades II-IV decreased from 40% to 28%, and grades III-IV from 19% to 11%.[25] Most patients received CNI-based prophylaxis, with a marked increase in ATG use over time (from 10% to 61%), and ATG was associated with significantly reduced acute GVHD in multivariable analysis. Although survival after acute GVHD improved over time, from 38% to 45%, it is still associated to significant mortality. Our study differs from the EBMT cohort by including haploidentical and mismatched (MMUD/MMRD) donors, the near-universal use of PBSC grafts, and a predominant reliance on PTCy rather than ATG. Despite these higher-risk features, acute GVHD incidences in our cohort were comparable (II-IV, 28%; III-IV, 7%), supporting the effectiveness of PTCy in mitigating acute GVHD even in inherently higher-risk settings.

A clinically relevant observation from our study is that despite the overall decline in acute GVHD, it continues to adversely affect survival, with particularly poor outcomes in MMUD/MMRD recipients and in those with grade III-IV GVHD, highlighting the need for improved approaches in this high-risk population.

The incidence of chronic GVHD has traditionally been high, historically ranging from 49%-56% in MRD to 70%-76% in MUD transplants, largely driven by PBSC grafts and CNI-based prophylaxis.[3–6] These earlier studies used older diagnostic and grading criteria of chronic GVHD, which may partly explain the high rates by classifying cases of late acute GVHD as chronic. More recent studies using contemporary NIH definitions report incidences between 35% and 49% with conventional GVHD prophylaxis.[16,22,26-28] In our real-world cohort, chronic GVHD incidence was lower than in historical series,

306 with 2-year overall and moderate-to-severe rates of 36.7% and 23.5%, respectively,
307 despite the widespread use of PBSC grafts. Rates were consistent across donor types,
308 including mismatched and haploidentical settings in which PTCy was predominantly
309 administered. These reduced incidences are likely attributable to the extensive use of
310 PTCy (and, to a lesser extent, ATG, less frequently used) consistent with multiple
311 studies showing the benefits of these strategies in lowering chronic GVHD.[8-18]

312 Our findings also align with those from a recent single-center retrospective
313 study at the Fred Hutchinson Cancer Center, which evaluated chronic GVHD requiring
314 systemic immunosuppressive therapy in 3,066 survivors (2005–2019).[29] In the most
315 recent period, the incidence was approximately 26%, similar to the rates observed in
316 our cohort. Notably, only ~20% of patients in that study received ATG, PTCy, or T-cell–
317 depleting prophylaxis, and the authors could not clearly identify the reasons for the
318 observed decline, attributing it to multiple evolving factors over the past decades.

319 One of the major strengths of our study is its ability to capture real-world
320 clinical practice regarding GVHD prophylaxis and the actual incidence of GVHD across a
321 large national cohort in a country with one of the highest alloHSCT volumes in Europe.
322 Despite expected inter-center variability, participating institutions consistently
323 adhered to contemporary diagnostic and grading criteria for acute and chronic GVHD.
324 Our findings also reveal notable differences between Spanish practice and the recently
325 published EBMT expert recommendations.[30] While EBMT considers both PTCy and ATG
326 acceptable options in MUD and MMUD/MMRD settings, our data show a clear national
327 preference for PTCy. Moreover, although EBMT does not formally recommend PTCy
328 for MRD transplantation, its use in this setting was frequent and increasingly

widespread. Another important observation is the low incidence of GVHD in HLA-mismatched and haploidentical recipients, likely attributable to extensive PTCy use, prompting reconsideration of traditionally recognized GVHD risk factors such as HLA disparities. Collectively, these insights may inform the design of future clinical trials in GVHD prevention and treatment.

Our study has limitations. Its observational, descriptive design precludes robust multivariable analyses of GVHD risk factors or definitive comparisons between prophylaxis strategies. The associations observed between prophylaxis approaches and GVHD outcomes should not be interpreted as evidence of superiority but rather as reflections of current practice patterns and their real-world correlates.

In conclusion, in this large real-world cohort, PTCy-based prophylaxis emerged as the predominant strategy across donor types and was associated with reduced incidences of both acute and chronic GVHD, particularly severe forms, compared with historical CNI-based regimens. While this represents a meaningful advance, it also introduces challenges for future clinical trial design and underscores the need for expanded collaborative efforts. The reduced incidence of GVHD may also limit future opportunities to identify biomarkers for risk stratification and early detection. Importantly, despite the overall decline in GVHD rates, acute GVHD continues to exert a substantial negative impact on post-transplant survival, reinforcing the need for continued refinement of prophylactic and therapeutic strategies.

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Authorship Contributions

CM, LBM, MJ, CS conceived and designed the study, interpreted the statistical results, and drafted the paper; MQS conducted the statistical analysis and interpreted the results; PB, SF, and AC provided assistance with the database's data management and the administrative aspects of the multicentre study; JM, JS, LLC, EPL, MJP, IGC, MSL, AB, FP, CC, MJJL, PAG, AS, IH, and TZ were the principal investigators at the participating centers, provided the patient data, and critically reviewing and approving the manuscript.

Data Sharing Statement: For original data, please contact Carmen Martínez (cmarti@clinic.cat)

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FIGURES LEGENDS

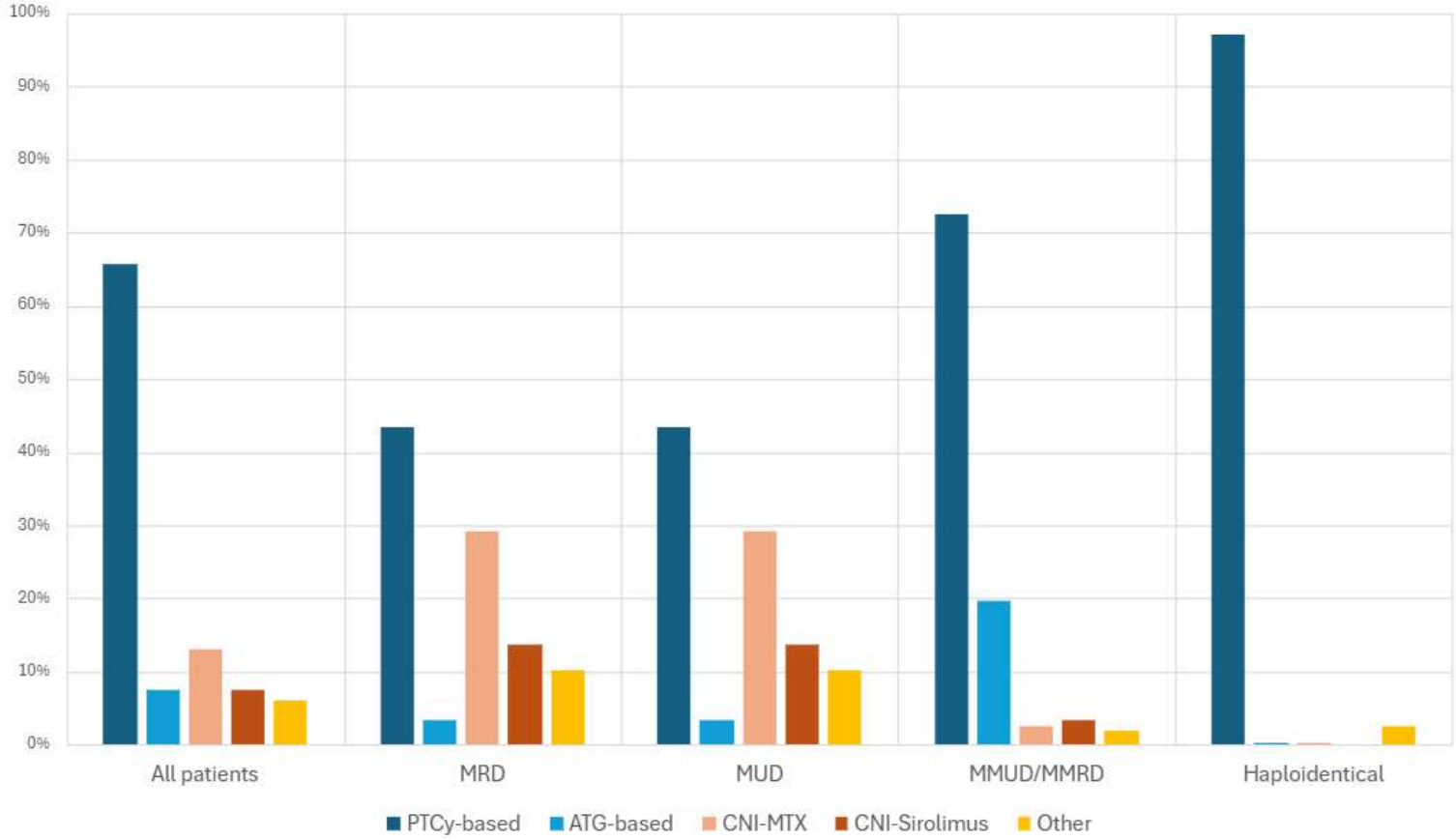
Figure 1. Type of GVHD prophylaxis according to the type of donor.

Figure 2. Cumulative incidence of grade II-IV (A) and grade III-IV (B) acute GVHD for the entire cohort.

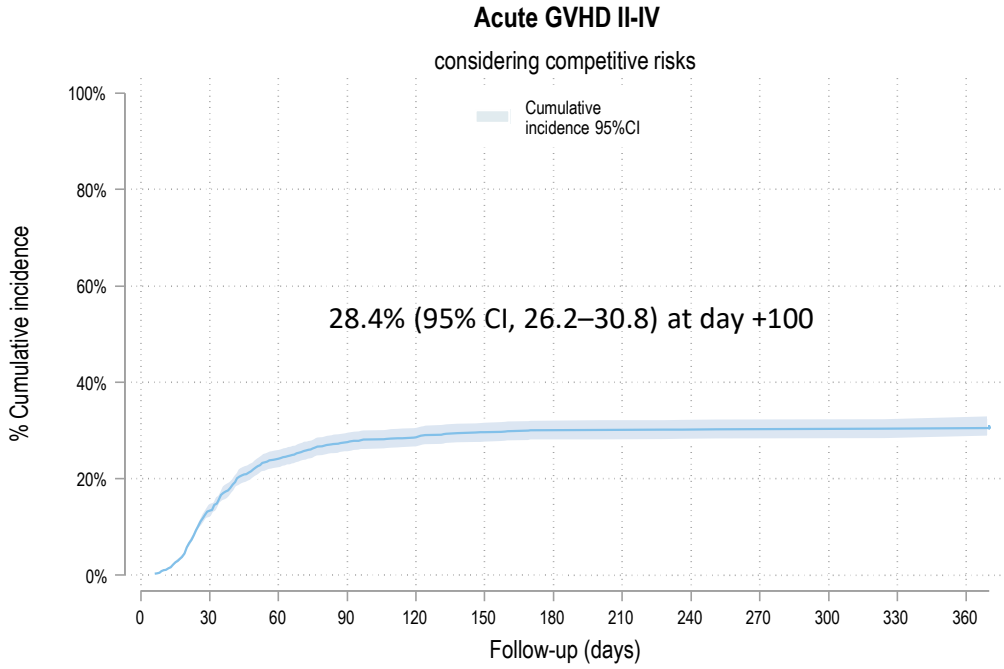
Figure 3. Cumulative incidence of acute GVHD according to the type of GVHD prophylaxis (A, grade II-IV; B, grade III-IV).

Figure 4. Cumulative incidence of global (A) and moderate-severe (B) chronic GVHD for the entire cohort.

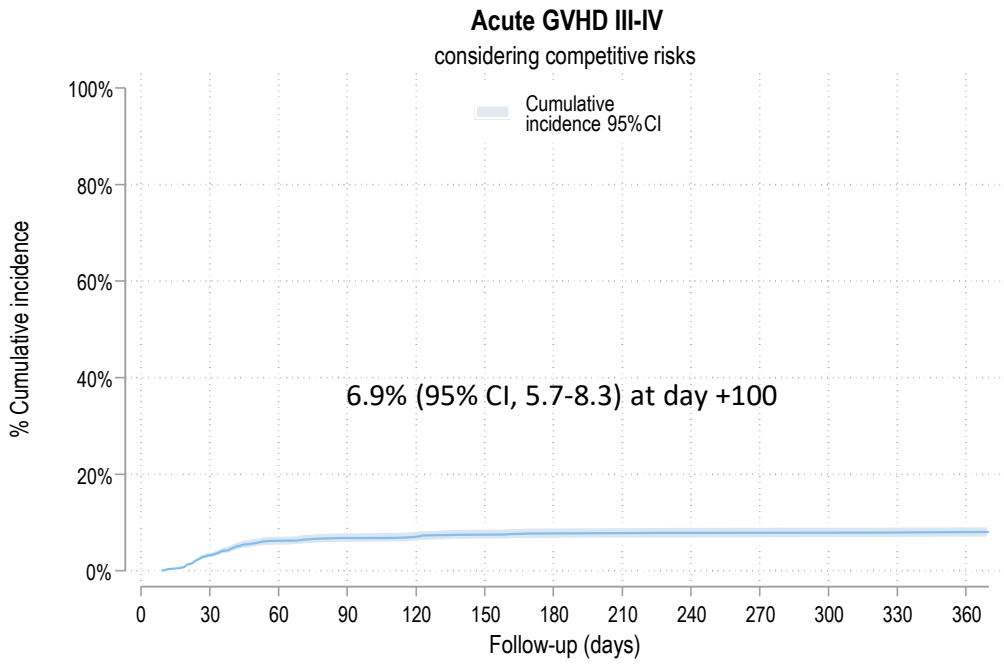
Figure 5. Cumulative incidence of chronic GVHD according to the type of GVHD prophylaxis (A, global; B, moderate-severe).

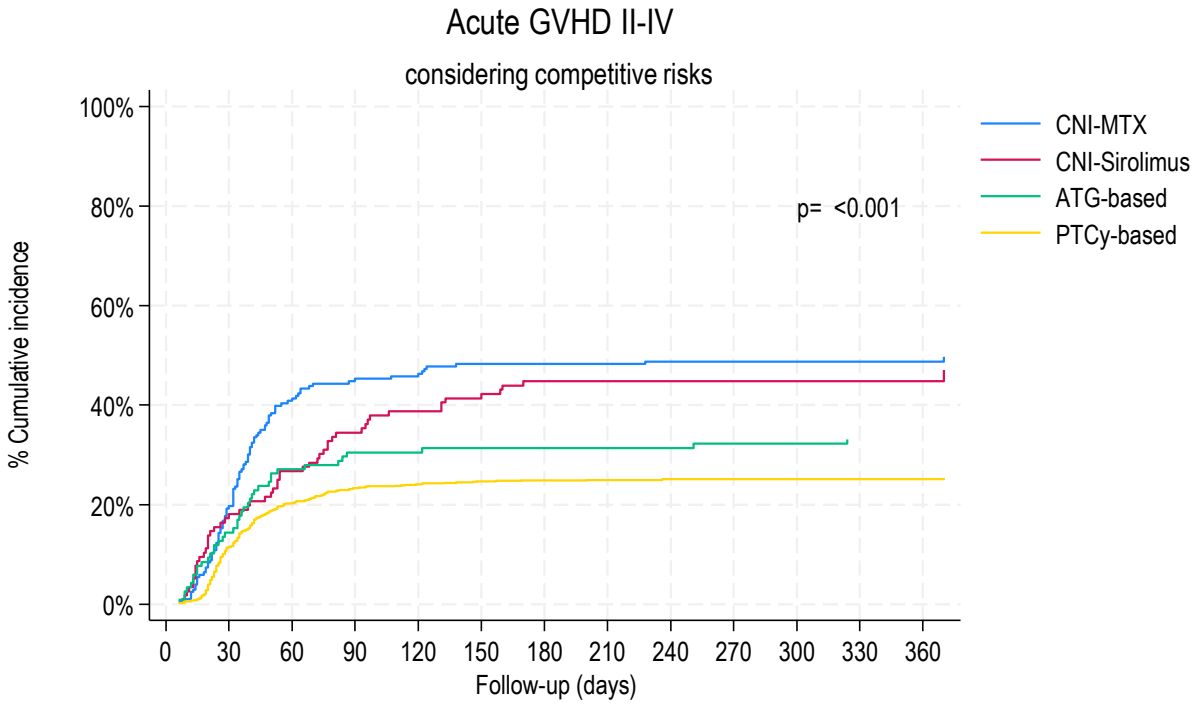

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
All patients
MRD
MUD
MMUD/MMRD
Haploidentical
PTCy-based
ATG-based
CNI-MTX
CNI-Sirolimus
Other

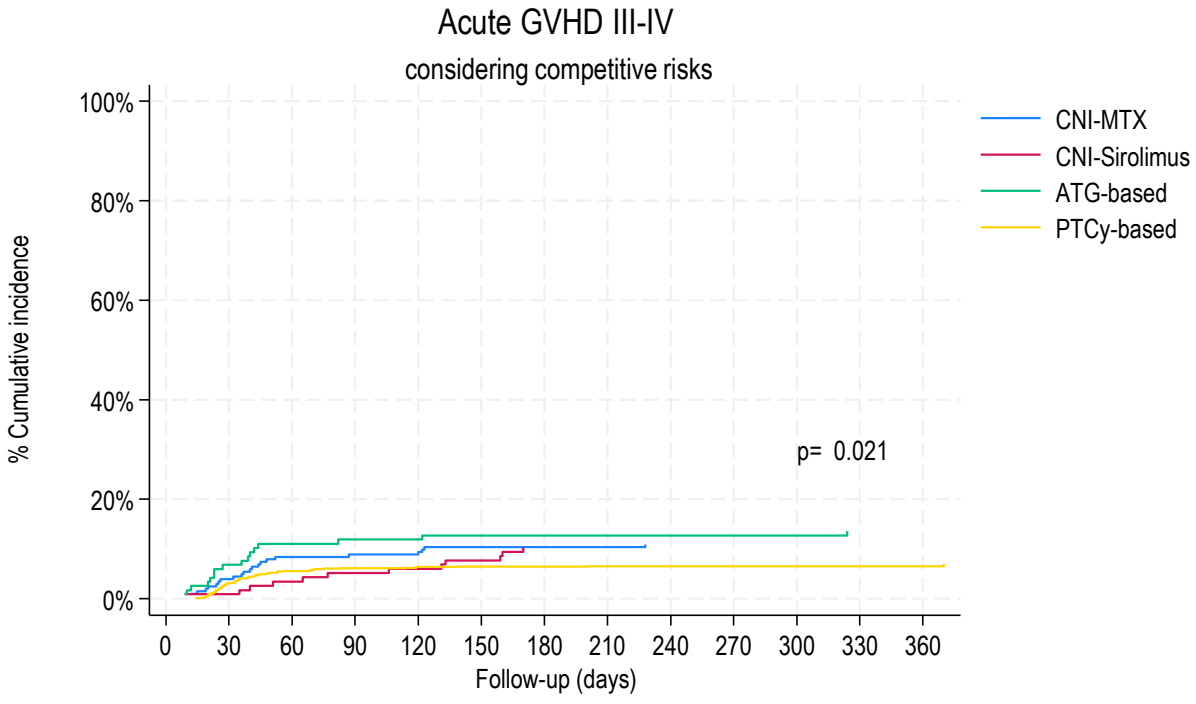
A

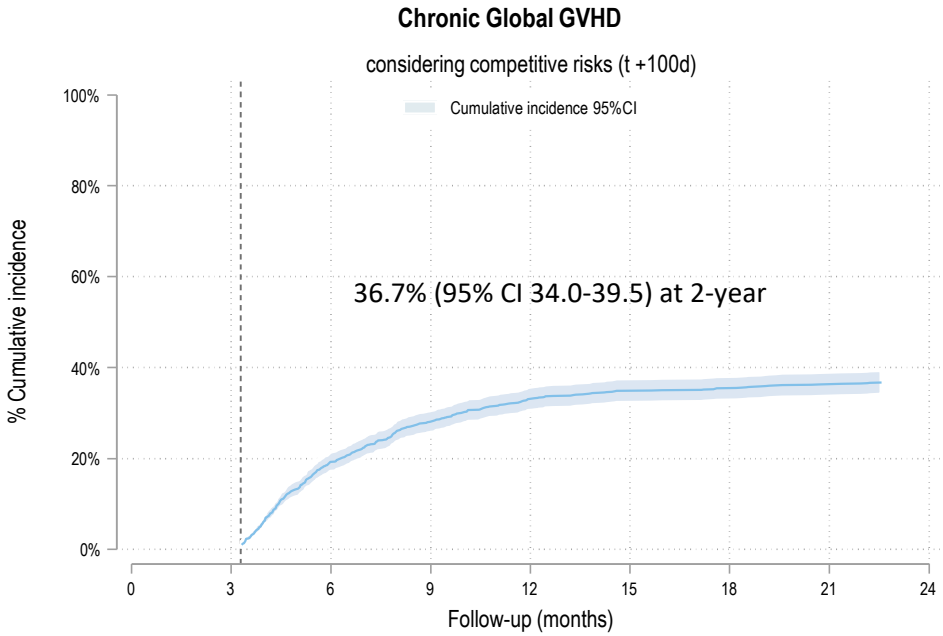

Acute GVHD II-IV
considering competitive risks
Cumulative incidence 95%CI
28.4% (95% CI, 26.2–30.8) at day +100
% Cumulative incidence
100%
80%
60%
40%
20%
0%
0
30
60
90
120
150
180
210
240
270
300
330
360
Follow-up (days)

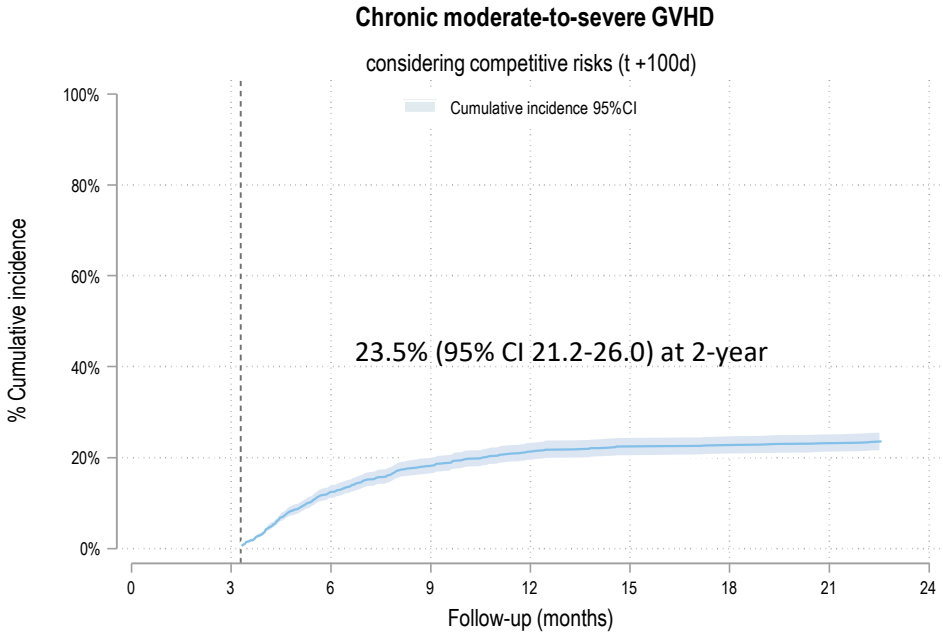
B

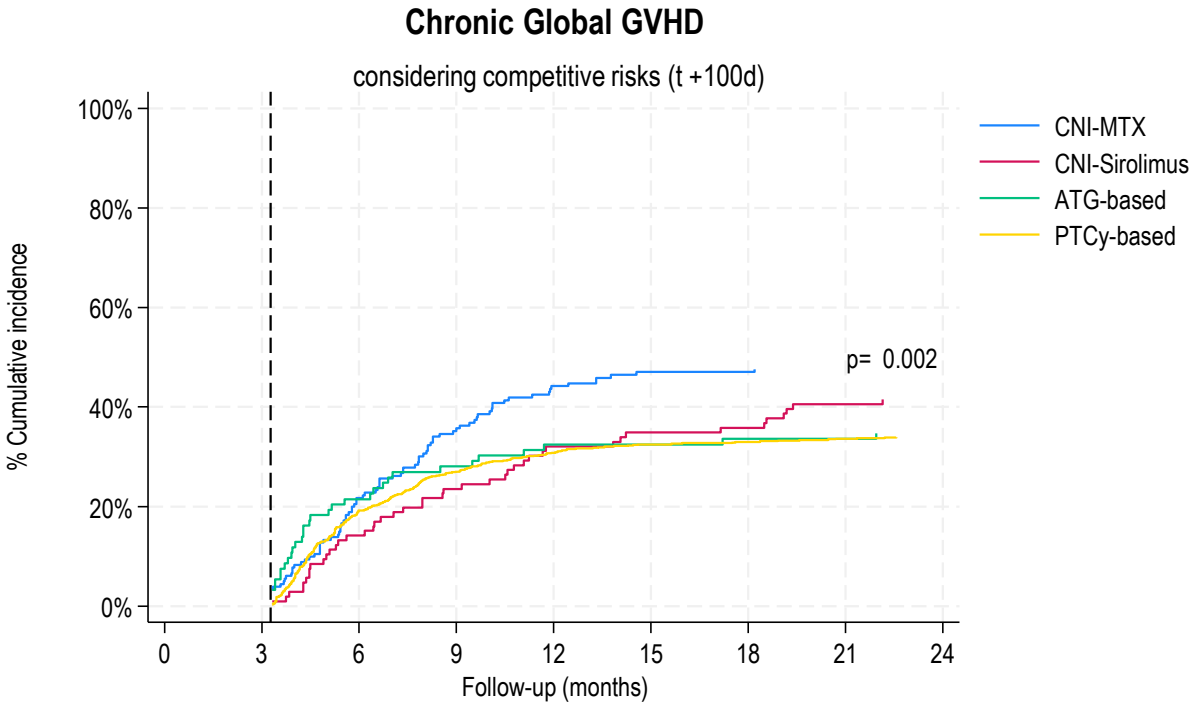

Acute GVHD III-IV
considering competitive risks
Cumulative incidence 95%CI
6.9% (95% CI, 5.7-8.3) at day +100
% Cumulative incidence
100%
80%
60%
40%
20%
0%
0
30
60
90
120
150
180
210
240
270
300
330
360
Follow-up (days)

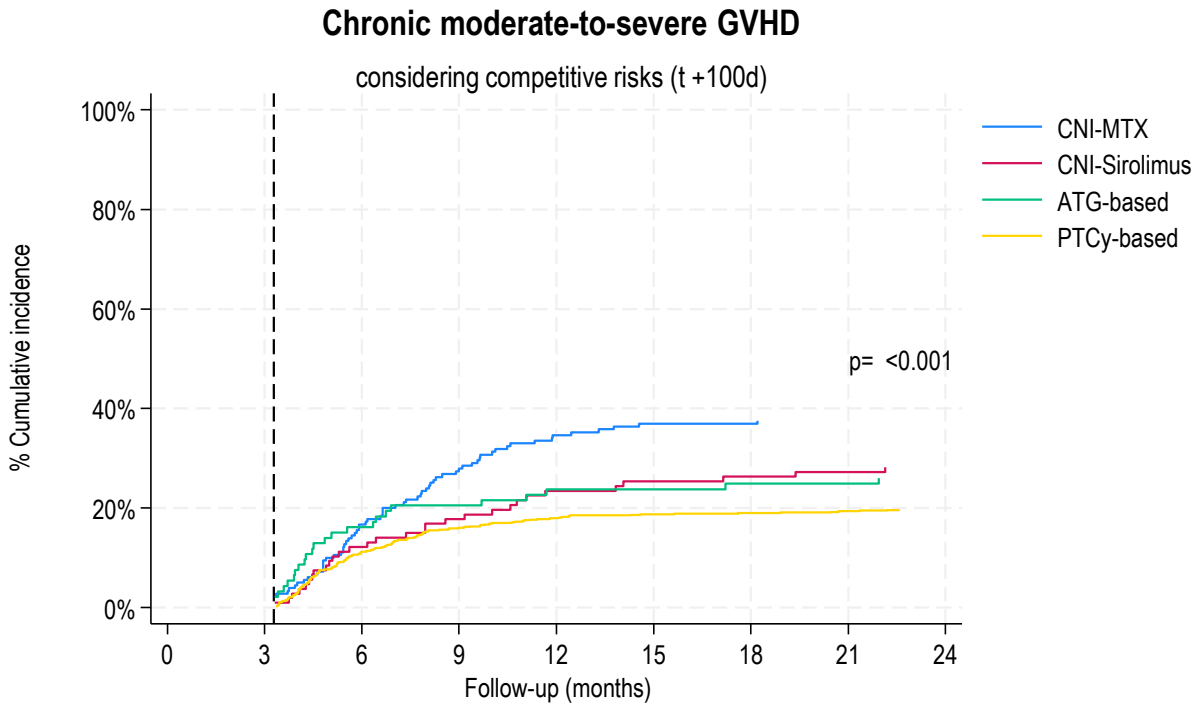

Acute GVHD II-IV
considering competitive risks
% Cumulative incidence
100%
80%
60%
40%
20%
0%
p= <0.001
0
30
60
90
120
150
180
210
240
270
300
330
360
Follow-up (days)
CNI-MTX
CNI-Sirolimus
ATG-based
PTCy-based


Acute GVHD III-IV
considering competitive risks
% Cumulative incidence
100%
80%
60%
40%
20%
0%
p= 0.021
0
30
60
90
120
150
180
210
240
270
300
330
360
Follow-up (days)
CNI-MTX
CNI-Sirolimus
ATG-based
PTCy-based


Chronic Global GVHD
considering competitive risks (t +100d)
Cumulative incidence 95%CI
% Cumulative incidence
100%
80%
60%
40%
20%
0%
36.7% (95% CI 34.0-39.5) at 2-year
0
3
6
9
12
15
18
21
24
Follow-up (months)


Chronic moderate-to-severe GVHD
considering competitive risks (t +100d)
Cumulative incidence 95%CI
% Cumulative incidence
100%
80%
60%
40%
20%
0%
23.5% (95% CI 21.2-26.0) at 2-year
0
3
6
9
12
15
18
21
24
Follow-up (months)


Chronic Global GVHD
considering competitive risks (t +100d)
% Cumulative incidence
100%
80%
60%
40%
20%
0%
0
3
6
9
12
15
18
21
24
Follow-up (months)
p= 0.002
CNI-MTX
CNI-Sirolimus
ATG-based
PTCy-based


Chronic moderate-to-severe GVHD
considering competitive risks (t +100d)
% Cumulative incidence
100%
80%
60%
40%
20%
0%
0
3
6
9
12
15
18
21
24
Follow-up (months)
p= <0.001
CNI-MTX
CNI-Sirolimus
ATG-based
PTCy-based

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.pdf
- Table1.pdf
- Table2.pdf
- Table3.pdf