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DIAGNOSIS AND TREATMENT OF GVHD

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INTRODUCTION

Around 50% of patients who undergo a hematopoietic stem cell transplant (HSCT) develop graft-versus-host disease (GVHD), with varying degrees of clinical severity and mortality rates of up to 20%[1,2]. The current guidelines will focus on the diagnosis, staging, grading, prophylaxis, and treatment of acute (aGVHD) and chronic GVHD (cGVHD).

DIAGNOSIS OF ACUTE GRAFT-VERSUS-HOST DISEASE (AGVHD)

The main risk factors for aGHVD are: HLA-mismatch between donor and recipient; gender disparity between donor and patient; conditioning regimen intensity; prophylaxis regimen used; progenitor stem cell source (peripheral blood > bone marrow > umbilical blood cord[3].

The skin, gastrointestinal (GI) tract, and liver are the most commonly affected organs in aGVHD. End-organ manifestations are characterized by a maculopapular rash (skin), nausea, vomiting, anorexia, and diarrhea (gut), and elevated bilirubin, canalicular enzyme, and, less often, transaminase levels (liver)[4,5].

ACUTE GVHD (AGVHD) STAGING AND CLASSIFICATION

The Mount Sinai Acute GVHD International Consortium (MAGIC) has recently allowed for a better standardization of the criteria for classification and data collection related to aGVHD[6]. It is currently regarded as the most appropriate method for the diagnosis, staging, and grading of aGVHD[6,7], as shown in tables 1 and 2, below:

TABLE 1 - MAGIC Target Organ aGVHD Staging

Stage	Skin (erythema)	Liver (bilirubin)	Upper GI tract	Lower GI tract (stool output per day)
0	No active rash	<2mg/dL	No or intermittent nauseaa, vomiting or anorexia	Adult: < 500 ml/day or <3 episodes/day Child: < 10 ml/kg/day or <4 episodes/dayb
1	Maculopapular rash <25% BSA	2-3 mg/dL	Persistent nausea, vomiting or anorexiaa	Adult: 500–999ml/day or 3–4 episodes/day Child: 10–19.9 ml/kg/day or 4–6 episodes/day
2	Maculopapular rash 25 – 50% BSA	3.1-6 mg/dL		Adult: 1000–1500 ml/day or 5–7 episodes/day Child: 20 – 30 ml/kg/day or 7–10 episodes/day

3	Maculopapular rash > 50% BSA	6.1-15 mg/dL		Adult: >1500 ml/day or >7 episodes/day Child: > 30 ml/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) plus bullous formation and desquamation > 5% BSA	>15 mg/dL		Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).

a. A diagnosis of aGVHD is suspected when anorexia is associated with weight loss, nausea lasting for at least 3 days, or accompanied by vomiting ≥ 2 episodes/day for at least 2 days; b. one episode of diarrhea corresponds to approximately 200 ml of stool volume in adults and 3ml/kg in children (< 50 kg).
MAGIC: Mount Sinai Acute GVHD International Consortium. BSA: body surface area. Adapted from A.C. Harris *et al.* /Biol Blood Marrow Transplant 22 (2016) 4e10.

TABLE 2 – MAGIC Overall Clinical Grading of aGVHD

Overall grading	Skin (erythema)	Liver (Bilirubin)	Upper GI tract	Lower GI tract (stool output per day)
0	0	0	0	0
I	1-2	0	0	0
II	3	1	1	1
III	0-3	2-3	0-1	2-3
IV	4	4	0-1	4

Magic: Mount Sinai Acute GVHD International Consortium
Adapted from: A.C. Harris *et al.* / Biol Blood Marrow Transplant 22 (2016) 4e10.

GVHD PROPHYLAXIS8

Table 3 depicts the main GVHD prophylaxis regimens used in myeloablative, non-myeloablative, and reduced-intensity conditioning allogeneic HSCT, including peripheral blood stem cell (PBSC) and haploidentical transplants, along with their corresponding levels of evidence and grades of recommendation.

TABELA 3 - Main GVHD prophylaxis regimens used, with levels of evidence and grades of recommendation

Type of allo-HSCT	Prohylaxis Regimen	Level of Evidence
MA allo-HSCT from related and unrelated donors	Calcineurin inhibitor and Methotrexate (MTX)9–15	Level 1a, grade of recommendation A
	Calcineurin inhibitor and Mycophenolate Mofetil (MMF)14–19	Level 1a, grade of recommendation B
	High-Dose Post-Transplant Cyclophosphamide (50 mg/kg on D+3 and D+4) 20–24	Level 2b, grade of recommendation C
RIC and NMA allo-HSCT from related donors	Calcineurin inhibitor and MMF25	Level 4, grade of recommendation C
HLA-identical allo-HSCT from related and unrelated donors using PBSC as stem cell source	Rabbit Antithymocyte Globulin (rATG) < 6 mg/kg26–33	Level 1a, grade of recommendation A
Haploidentical allo-HSCT – Baltimore protocol	High-Dose Post-Transplant Cyclophosphamide (50 mg/kg on D+3 and D+4) plus a calcineurin inhibitor and MMF34–36	Level 2b, grade of recommendation B
Haploidentical allo-HSCT – Beijing protocol	High-Dose rATG (10 mg/kg), MMF, calcineurin inhibitor, and MTX37	Level 2b, grade of recommendation B

allo-HSCT: allogeneic hematopoietic stem cell transplant; MA: myeloablative; NMA: non-myeloablative; RIC: reduced-intensity conditioning; HLA: human leukocyte antigen; PBSC: peripheral blood stem cells.

TREATMENT OF AGVHD

Grade I aGVHD: optimize prophylaxis regimen, adjusting for calcineurin inhibitor trough levels, and add topical agents (corticosteroids or tacrolimus). No systemic immunosuppression is recommended[38] – Level of evidence 1b, Grade of recommendation A.

Grade II-IV aGVHD: start systemic treatment with methylprednisolone (MP) at a dose of 2mg/kg/day or its prednisone equivalent[39] – **Level of evidence 1a, Grade of recommenation A**. Concomitant calcineurin inhibitor (cyclosporine or tacrolimus) prophylaxis should not be withdrawn. For less severe forms (grade IIa aGVHD), start MP at a dose of 0.5-1mg/kg/day, escalating up to 2 mg/kg if worsening occurs after 72h[40] – Level of evidence 1b, Grade of recommendation A. Non-absorbable glucocorticoids (beclomethasone and budesonide) have also been used in the treatment of mild upper or lower GI aGVHD (500-1000 ml/stool output/day) as an adjuvant to systemic corticosteroids[41,42] – **Level of evidence 1b, Grade of recommendation A.**

SECOND-LINE TREATMENT OF GRADE II-IV AGVHD

Second-line treatment is recommended in case of aGVHD progression within the first three days (72h) or lack of improvement after 5-7 days after initial therapy with MP 2mg/kg/day[8] – Level of evidence 5, Grade of recommendation D. Studies on the second-line treatment of aGVHD are highly heterogeneous, with hardly comparable results, great drug and interrater variability, as well as variability across centers. Since no superiority of one agent over another has been proven to date, the choice of the most appropriate approach should be individualized and dependent upon the following factors: previous therapy, drug interaction, availability, accessibility, and center expertise[8] – **Level of evidence 2b, Grade of recommendation C**. Table 4 shows the main treatment options for the second-line treatment of grade II-IV aGVHD.

TABLE 4 - Second-line therapy for grade II-IV aGVHD, with levels of evidence and grades of recommendation

MMF	Level of evidence 2b, Grade of recommendation C43–46	Complete Response (CR) and Partial Response (PR) rates of up to 77% in 6 months.
Extracorporeal Photopheresis (ECP)	Level of evidence 2a, Grade of recommendation B47–58	Overall response rates (ORR) of 84% in aGVHD of the skin and 65% in that of the gut
ATG	Level of evidence 2b, Grade of recommendation C59,60	ORR between 20% and 50%, particularly in aGHVD of the skin
Basiliximab	Level of evidence 2b, Grade of recommendation B61,62	Response rates of approximately 80%, with an overall survival of 30% at 5 years
Infliximab and Etarnecept	Level of evidence 2b, Grade of recommendation C63	ORR of approximately 70%, particularly in aGVHD of the gut
Ruxolitinib	Level of evidence 1b, Grade of recommendation A64–69	REACH2* phase III study showed an ORR of 62% at 28 days, compared to a 39% ORR in the control group

MMF: mycophenolate mofetil; ATG: antithymocyte globulin; GVHD: graft-versus-host disease.

CHRONIC GRAFT-VERSUS-HOST DISEASE (CGVHD)

With a prevalence of 30-70% among allogeneic HSCT recipients, cGVHD remains the main cause of long-term post-transplant morbidity and mortality in this population[70–72]. The cumulative incidence of cGVHD at 2 years in patients undergoing related or unrelated, bone marrow or peripheral blood stem cell allogeneic HSCT, as defined by the National Institute of Health (NIH) criteria, was 34%[73].

DIAGNOSIS OF CGVHD AND ITS DIFFERENTIATION FROM AGVHD

The 2014 NIH Consensus recognized two main categories of (acute and chronic) GVHD. The clinical manifestations, and not the actual time of onset of symptoms, are the basis for classifying a case as of acute or chronic GVHD[73]. Table 5 depicts the established categories for acute and chronic GVHD.

TABLE 5 - Acute and Chronic GVHD Categories

Category		Time of onset	aGVHD	cGVHD
aGVHD	Classic	£100 days	Yes	No
	Persistent/Recurrent/ Late Acute	> 100 days	Yes	No
cGVHD	Classic (De Novo/Quiescent/Progressive)	No limit	No	Yes
	Overlap	No limit	Yes	Yes

aGVHD: persistent (previously unresolved aGVHD); recurrent (previously resolved aGVHD); late acute (without prior aGVHD); classic and overlap cGVHD: De Novo (without prior aGVHD); quiescent (previously resolved aGVHD); progressive (previously unresolved aGVHD)

CLINICAL SCORING SYSTEM BY TARGET ORGAN

The target organs comprised by the cGVHD scoring system include the skin, mouth, eyes, GI tract, liver, lungs, joints, fasciae, and urogenital (UG) tract. Each organ or body part receives a score within a 4-point (0-3) scale, in which "0" represents absence of involvement and "3" reflects severe involvement74. Table 6 displays each of the cGVHD severity levels.

TABLE 6 - Chronic GVHD severity

Mild cGVHD Involvement of 1 or 2 organs AND organ score of 1 AND a lung score of 0
Moderate cGVHD ≥3 organs with a score of 1 OR at least 1 organ with a score of 2 OR a lung score of 1
Severe cGVHD At least one organ with a score of 3 OR a lung score of 2

cGVHD: chronic graft-versus-host disease.

The use of the 2014 NIH criteria for the diagnosis of cGVHD is both feasible and reliable in pediatric patients. However, specific adjustments in such criteria are needed to better assess the degree of lung and ocular involvement, since pulmonary function tests (PFTs) and Schirmer's test, respectively, are technically difficult to perform in children younger than 6 years of age[75,76].

TREATMENT OF CHRONIC GVHD (CGVHD)

The main criteria for initiating systemic treatment for cGVHD comprise: score >2 in at least one organ, involvement of three or more organs with score 1, lung score 1 or 2, and mild cGVHD with high-risk features (thrombocytopenia <100.000/mm3 and use of immunosuppressants at cGVHD diagnosis)77. The standard treatment consists of prednisone at a dose of 1mg/kg/day and cyclosporine[78,79]. **Level of evidence 1c, Grade of recommendation A.**

DEFINITION OF REFRACTORINESS TO SYSTEMIC TREATMENT

Progression of cGVHD after 2 weeks of systemic therapy (prednisone 1 mg/kg/day), stable disease while on prednisone (>0.5 mg/kg/day) for 4-8 weeks, or inability to reduce the dose of prednisone to < 0.5 mg/kg/day80. **Level of evidence 5, Grade of recommendation D.**

INDICATIONS FOR SECOND-LINE THERAPY OF CGVHD

Worsening of cGVHD manifestations in a primarily involved target organ, absence of any treatment response after one month, or inability to reduce the dose of prednisone to < 1 mg/kg/day within two months[79]. Table 7 depicts the main agents used in the second-line therapy of cGVHD.

TABLE 7 - Main agents used in the second-line therapy of cGVHD, with levels of evidence and grades of recommendation

Extracorporeal Photopheresis (ECP)	Level of evidence 1b, Grade of recommendation A57,81–85	Mucocutaneous manifestations, with complete response (CR) rates of > 80% and significant improvement of sclerotic cGVHD.
Mycophenolate Mofetil	Level of evidence 4, Grade of recommendation B86,87	Overall response rates (ORR) vary between 23% and 79% in several case series
Sirolimus	Level of evidence 4, Grade of recommendation B88–90	ORR varying between 63% and 81% in several case series
Rituximab	Level of evidence 2b, Grade of recommendation B80,91,92	Mucocutaneous and musculoskeletal manifestations, with an ORR of approximately 70%
Imatinib	Level of evidence 2b, Grade of recommendation B92,93	Cutaneous, ocular, and gut manifestations, with an ORR between 50% and 80%
Methotrexate	Level of evidence 4, Grade of recommendation B94,95	ORR varying between 58.8% and 71% in most case series
Ibrutinib	Level of evidence 2b, Grade of recommendation B96,97	ORR of 67%, with a 21% CR rate
Ruxolitinib	Level of evidence 4, Grade of recommendation C98	ORR of 57%, with a 1-year overall survival of 81%

cGVHD: chronic graft-versus-host disease.

REFERENCES

1. Appelbaum FR. Hematopoietic-Cell Transplantation at 50. *N. Engl. J. Med*. v. 357, n. 15, p. 1472-1475, 2007.

2. CIBMTR. Center for International Blood and Marrow Transplant Research. *Current uses and outcomes of hematopoietic stem cell transplantation* 2014: summary slides.

3. Flowers MED, Inamoto Y, Carpenter PA, et al. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. *Blood*. v.117, n.11, p. 3214-3219,

4. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. v. 18, n.4, p. 295-304, 1974.

5. Schwartz J. Severe gastrointestinal bleeding after hematopoietic cell transplantation, 1987–1997: incidence, causes, and outcome. *Am. J. Gastroenterol*. v.96, n.2, p. 385-393, 2001.

6. Harris AC, Young R, Devine S, *et al*. International, Multicenter Standardization of Acute Graft-versus-Host Disease Clinical Data Collection: A Report from the Mount Sinai Acute GVHD International Consortium. *Biol. Blood Marrow Transplant*.v.22, n.1, p. 4-10, 2016.

7. Schoemans HM, Lee SJ, Ferrara JL, *et al.* EBMT–NIH–CIBMTR Task Force position statement on standardized terminology & guidance for graft-versus-host disease assessment. *Bone Marrow Transplant*. v.53. n. 11, p. 2018.

8. Penack O, Marchetti M, Ruutu T, et al. Prophylaxis and management of graft versus host disease after stem-cell transplantation for haematological malignancies: updated consensus recommendations of the European Society for Blood and Marrow Transplantation. *Lancet Haematol*. v. 7, n.2, p. 157-167, 2020.

9. Nash RA, Piñeiro LA, Storb R, *et al.* FK506 in combination with methotrexate for the prevention of graft-versus-host disease after marrow transplantation from matched unrelated donors. *Blood*. v. 88, n. 9, p. 3634-41, 1996.

10. Przepiorka D, Ippoliti C, Khouri I, *et al.* Tacrolimus and minidose methotrexate for prevention of acute graft-versus-host disease after matched unrelated donor marrow transplantation. *Blood*. v.88, n. 11, p. 4383-9, 1996.

11. Ratanatharathorn V, Nash RA, Przepiorka D, *et al.* Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood*. v.92, n.7, p. 2303-2314, 1998.

12. Nash RA, Antin JH, Karanes C, *et al.* Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood*. v.96, n.6, p. 2062-2068, 2000.

13. Inamoto Y, Flowers MED, Appelbaum FR, *et al.* A Retrospective Comparison of Tacrolimus versus Cyclosporine with Methotrexate for Immunosuppression after Allogeneic Hematopoietic Cell Transplantation with Mobilized Blood Cells. *Biol. Blood Marrow Transplant.* v.17, n.7, p. 1088-1092, 2011.

14. Ram R, Yeshurun M, Vidal L, Shpilberg O, Gafter-Gvili A. Mycophenolate mofetil vs. methotrexate for the prevention of graft-versus-host-disease – Systematic review and meta-analysis. Leuk. *Res*. v.38, n.3, p. 352-360, 2014.

15. Bolwell B, Sobecks R, Pohlman B, *et al.* A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. *Bone Marrow Transplant*. v.34, n.7, p. 621-625, 2004.

16. Perkins J, Field T, Kim J, et al. A Randomized Phase II Trial Comparing Tacrolimus and Mycophenolate Mofetil to Tacrolimus and Methotrexate for Acute Graft-versus-Host Disease Prophylaxis. *Biol. Blood Marrow Transplant*. v.16, n.7, 2010.

17. Kharfan-Dabaja M, Mhaskar R, Reljic T, *et al.* Mycophenolate mofetil versus methotrexate for prevention of graft-versus-host disease in people receiving allogeneic hematopoietic stem cell transplantation. *Cochrane Database Syst. Rev.* 2014;

18. Hamilton BK, Rybicki L, Dean R, *et al.* Cyclosporine in combination with mycophenolate mofetil versus methotrexate for graft versus host disease prevention in myeloablative HLA-identical sibling donor allogeneic hematopoietic cell transplantation. *Am. J. Hemato*l v. 90, n.2, p. 144-148.

19. Chhabra S, Liu Y, Hemmer MT, *et al.* Comparative Analysis of Calcineurin Inhibitor–Based Methotrexate and Mycophenolate Mofetil–Containing Regimens for Prevention of Graft-versus-Host Disease after Reduced-Intensity Conditioning Allogeneic Transplantation. *Biol. Blood Marrow Transplant*. v.25, n.1, p. 73-85,. 2019.

20. Luznik L, Bolaños-Meade J, Zahurak M, *et al.* High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. Blood. v.115, n. 16, p. 3497-3505, 2010.

21. Kanakry CG, O'Donnell P V., Furlong T, *et al.* Multi-Institutional Study of Post-Transplantation Cyclophosphamide As Single-Agent Graft-Versus-Host Disease Prophylaxis After Allogeneic Bone Marrow Transplantation Using Myeloablative Busulfan and Fludarabine Conditioning. *J. Clin. Oncol.* v.32, p. 31, p. 3497-3505, 2014.

22. Mielcarek M, Furlong T, O'Donnell P V., *et al.* Posttransplantation cyclophosphamide for prevention of graft-versus-host disease after HLA-matched mobilized blood cell transplantation. *Blood*. 127, . 11, p. 1502-1508, 2016.

23. Jacoby E, Chen A, Loeb DM, *et al.* Single-Agent Post-Transplantation Cyclophosphamide as Graft-versus-Host Disease Prophylaxis after Human Leukocyte Antigen–Matched Related Bone

Marrow Transplantation for Pediatric and Young Adult Patients with Hematologic Malignancies. Biol. *Blood Marrow Transplant*. v.22, n.1, p. 112-118, 2016.

24. Kanakry CG, Tsai H-L, Bolaños-Meade J, *et al.* Single-agent GVHD prophylaxis with posttransplantation cyclophosphamide after myeloablative, HLA-matched BMT for AML, ALL, and MDS. *Blood*. v.124, n.25, p. 3817-3827, 2014.

25. McSweeney PA, Niederwieser D, Shizuru JA, *et al.* Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood*. v.97, n.11. p. 3390-3400 2001.

26. Kröger N, Solano C, Wolschke C, *et al.* Antilymphocyte Globulin for Prevention of Chronic Graft-versus-Host Disease. *N. Engl. J. Med.* v.374, n.1, p. 43-53, 2016.

27. Arai Y, Jo T, Matsui H, Kondo T, Takaori-Kondo A. Efficacy of antithymocyte globulin for allogeneic hematopoietic cell transplantation: a systematic review and meta-analysis. Leuk. *Lymphoma*. v.58, n.8, p. 1840-1848, 2017.

28. Theurich S, Fischmann H, Shimabukuro-Vornhagen A, *et al.* Polyclonal anti-thymocyte globulins for the prophylaxis of graft-versus-host disease after allogeneic stem cell or bone marrow transplantation in adults. *Cochrane Database Syst. Rev.* 2012;

29. Bacigalupo A, Lamparelli T, Bruzzi P, *et al.* Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood*. v.98, n.1o, p. 2942-2947, 2001.

30. Finke J, Bethge WA, Schmoor C, *et al.* Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol.* v. 10, n.9, p. 855-864, 2009.

31. Socié G, Schmoor C, Bethge WA, *et al.* Chronic graft-versus-host disease: long-term results from a randomized trial on graft-versus-host disease prophylaxis with or without anti–T-cell globulin ATG-Fresenius. *Blood*. v. 117, n.23, p.8375- 6382.

32. Walker I, Panzarella T, Couban S, *et al.* Pretreatment with anti-thymocyte globulin versus no anti-thymocyte globulin in patients with haematological malignancies undergoing haemopoietic cell transplantation from unrelated donors: a randomised, controlled, open-label, phase 3, multicentre trial. *Lancet Oncol.* v.17, n. 2, p. 164-173, 2016.

33. Baron F, Mohty M, Blaise D, *et al*. Anti-thymocyte globulin as graft- versus -host disease prevention in the setting of allogeneic peripheral blood stem cell transplantation: a review from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. *Haematologica*. v.102, n.2, p. 224-234, 2017.

34.O'Donnell P., Luznik L, Jones R., *et al.* Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. *Biol. Blood Marrow Transplant*. v.8, n.7, p. 377-386, 2002.

35. Luznik L, O'Donnell P V., Symons HJ, *et al.* HLA-Haploidentical Bone Marrow Transplantation for Hematologic Malignancies Using Nonmyeloablative Conditioning and High-Dose, Posttransplantation Cyclophosphamide. *Biol. Blood Marrow Transplant.* v.14, n.6, p. 641-650, 2008.

36. Kasamon YL, Bolaños-Meade J, Prince GT, *et al.* Outcomes of Nonmyeloablative HLA-Haploidentical Blood or Marrow Transplantation With High-Dose Post-Transplantation Cyclophosphamide in Older Adults. *J. Clin. Oncol.* v.33, n.28, p. 3152-3161, 2015.

37. Lu D-P, Dong L, Wu T, *et al.* Conditioning including antithymocyte globulin followed by unmanipulated HLA-mismatched/haploidentical blood and marrow transplantation can achieve comparable outcomes with HLA-identical sibling transplantation. *Blood*. v. 107, n.8, p. 3065-3073, 2006.

38. Martin P, Schoch G, Fisher L, *et al.* A retrospective analysis of therapy for acute graft-versus-host disease: secondary treatment. *Blood*. v.77, n.8, p. 1821-1828, 1991.

39. Van Lint MT, Uderzo C, Locasciulli A, *et al.* Early treatment of acute graft-versus-host disease with high- or low- dose 6-methylprednisolone: A multicenter randomized trial from the Italian group for bone marrow transplantation. *Blood*. v.92, n.7, p. 2288, 2293, 1998.

40. Mielcarek M, Storer BE, Boeckh M, *et al.* Initial therapy of acute graft-versus-host disease with low-dose prednisone does not compromise patient outcomes. v.113, n.13, p. 2888-2894, 2009.

41. McDonald GB, Bouvier M, Hockenbery DM, *et al.* Oral beclomethasone dipropionate for treatment of intestinal graft-versus-host disease: A randomized, controlled trial. *Gastroenterology*. v. 115, n 1, p. 28-35, 1998.

42. Hockenbery DM, Cruickshank S, Rodell TC, *et al.* A randomized, placebo-controlled trial of oral beclomethasone dipropionate as a prednisone-sparing therapy for gastrointestinal graft-versus-host disease. *Blood*. v.109, n.10, p. 4557-4563, 2007.

43. Martin PJ, Rizzo JD, Wingard JR, *et al.* First- and Second-Line Systemic Treatment of Acute Graft-versus-Host Disease: Recommendations of the American Society of Blood and Marrow Transplantation. *Biol. Blood Marrow Transplant*. v.18, n. 8, p. 1150-1163, 2012.

44. Alousi AM, Weisdorf DJ, Logan BR, *et al.* Etanercept, mycophenolate, denileukin, or pentostatin plus corticosteroids for acute graft-versus-host disease: a randomized phase 2 trial from the Blood and Marrow Transplant Clinical Trials Network. *Blood*. v.114, n.3, p. 511, 517, 2009.

45. Furlong T, Martin P, Flowers MED, *et al.* Therapy with mycophenolate mofetil for refractory acute and chronic GVHD. *Bone Marrow Transplant*. v.44, n.11, p. 739-748, 2009.

46. Krejci M, Doubek M, Buchler T, *et al*. Mycophenolate mofetil for the treatment of acute and chronic steroid-refractory graft-versus-host disease. *Ann. Hematol.* v.84, n.10, p. 681-685, 2005.

47. Hart JW, Shiue LH, Shpall EJ, Alousi AM. Extracorporeal photopheresis in the treatment of graft- versus -host disease: evidence and opinion. *Ther. Adv. Hematol.* v.4, n.5, p. 320-334, 2013.

48. Greinix HT, Knobler RM, Worel N, *et al.* The effect of intensified extracorporeal photochemotherapy on long-term survival in patients with severe acute graft-versus-host disease. *Haematologica*. v.91, n.3, p. 405-8, 2006.

49. Modemann F, Ayuk F, Wolschke C, *et al.* Ruxolitinib plus extracorporeal photopheresis (ECP) for steroid refractory acute graft-versus-host disease of lower GI-tract after allogeneic stem cell transplantation leads to increased regulatory T cell level. *Bone Marrow Transplant*. 2020;

50. Schneiderman J. Extracorporeal photopheresis: cellular therapy for the treatment of acute and chronic graft-versus-host disease. *Hematology*. v.1, n.6, p. 639-644, 2017 .

51. Abu-Dalle I, Reljic T, Nishihori T, *et al.* Extracorporeal Photopheresis in Steroid-Refractory Acute or Chronic Graft-versus-Host Disease: Results of a Systematic Review of Prospective Studies. *Biol. Blood Marrow Transplant*. v.20, n.11, p. 2014.

52. Rafei H, Kharfan-Dabaja MA, Nishihori T. A Critical Appraisal of Extracorporeal Photopheresis as a Treatment Modality for Acute and Chronic Graft-Versus-Host Disease. *Biomedicines*. v.5, n.4, p. 60, 2017.

53. Shapiro RM, Antin JH. Therapeutic options for steroid-refractory acute and chronic GVHD: an evolving landscape. *Expert Rev. Hematol.* v.13, n. 5, p. 519-532, . 2020.

54. Malagola M, Cancelli V, Skert C, *et al.* Extracorporeal Photopheresis for Treatment of Acute and Chronic Graft Versus Host Disease. *Transplantation*. v. 100, n. 12, p. 147,155, 2016.

55. Jagasia M, Greinix H, Robin M, *et al.* Extracorporeal Photopheresis versus Anticytokine Therapy as a Second-Line Treatment for Steroid-Refractory Acute GVHD: A Multicenter Comparative Analysis. *Biol. Blood Marrow Transplant*. v.19, n.7, p. 1129-1133, 2013.

56. Greinix HT, Volc-Platzer B, Kalhs P, *et al.* Extracorporeal photochemotherapy in the treatment of severe steroid-refractory acute graft-versus-host disease: a pilot study. *Blood*. v.96, n.7, p. 2426-31, 2000.

57. Oarbeascoa G, Lozano ML, Guerra LM, *et al.* Retrospective Multicenter Study of Extracorporeal Photopheresis in Steroid-Refractory Acute and Chronic Graft-versus-Host Disease. *Biol. Blood Marrow Transplant*. v.26, n.4, p. 651-658, 2020.

58. Sestili S, Eder S, Belhocine R, *et al*. Extracorporeal photopheresis as first-line strategy in the treatment of acute graft-versus-host disease after hematopoietic stem cell transplantation: A single-center experience. *Cytotherapy*. v.22, n.8, p. 445-449, 2020.

59. MacMillan ML, Weisdorf DJ, Davies SM, *et al.* Early antithymocyte globulin therapy improves survival in patients with steroid-resistant acute graft-versus-host disease. Biol. *Blood Marrow Transplant*. v.8, n.1, p. 2002.

60. Carpenter PA, Sanders JE. Steroid-refractory graft-vs.-host disease: past, present and future. Pediatr. *Transplant*. v. 7, p. 19-31, 2003.

61. Massenkeil G, Rackwitz S, Genvresse I, *et al.* Basiliximab is well tolerated and effective in the treatment of steroid-refractory acute graft-versus-host disease after allogeneic stem cell transplantation. *Bone Marrow Transplant*. v.30, n.12, p. 899-903. 2002.

62. Funke VAM, de Medeiros CR, Setúbal DC, *et al.* Therapy for severe refractory acute graft-versus-host disease with basiliximab, a selective interleukin-2 receptor antagonist. *Bone Marrow Transplant*. v.37, n.10, p. 961-965, 2006.

63. Couriel D, Saliba R, Hicks K, *et al*. Tumor necrosis factor-α blockade for the treatment of acute GVHD. *Blood*. v. 104, n.3, p. 649-654, 2004.

64. Schroeder MA, Choi J, Staser K, DiPersio JF. The Role of Janus Kinase Signaling in Graft-Versus-Host Disease and Graft Versus Leukemia. *Biol. Blood Marrow Transplant*. v. 24, n.6, p. 1125-1134, 2018.

65. Choi J, Cooper ML, Alahmari B, *et al.* Pharmacologic Blockade of JAK1/JAK2 Reduces GvHD and Preserves the Graft-Versus-Leukemia Effect. *PLoS One*. v. 9, n.10, p. 109799, 2014.

66. Jagasia M, Perales M-A, Schroeder MA, *et al.* Ruxolitinib for the treatment of steroid-refractory acute GVHD (REACH1): a multicenter, open-label phase 2 trial. *Blood*. v. 135, n,20, p. 1739-1749, 2020.

67. FDA. *FDA approves ruxolitinib for acute graft-versus-host disease.* 2019;

68. Zeiser R, von Bubnoff N, Butler J, *et al.* Ruxolitinib for Glucocorticoid-Refractory Acute Graft-versus-Host Disease. *N. Engl. J. Med.* v.382, n.19, p. 1800-1810, 2020.

69. Uygun V, Karasu G, Daloğlu H, *et al.* Ruxolitinib salvage therapy is effective for steroid-refractory graft-versus-host disease in children: A single-center experience. Pediatr. *Blood Cancer.* v.67, n.4, 2020.

70. Lee SJ. Severity of chronic graft-versus-host disease: association with treatment-related mortality and relapse. *Blood*. v. 100, n. 2, p. 406-414, 2002.

71. Couriel D, Carpenter PA, Cutler C, *et al*. Ancillary Therapy and Supportive Care of Chronic Graft-versus-Host Disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: V. Ancillary Therapy and Supportive Care Working. *Biol. Blood Marrow Transplant.* v.12,n. 4, p. 375-396, 2006.

72. Arora M, Klein JP, Weisdorf DJ, *et al.* Arora M, Klein JP, Weisdorf DJ, *et al.* Chronic GVHD risk score: a Center for International Blood and Marrow Transplant Research analysis. Blood. 2011;117(24):6714-6720. *Blood*. v.118, n.26, p. 6992--6992, 2011.

73. Jagasia MH, Greinix HT, Arora M, *et al*. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group Report. Biol. *Blood Marrow Transplant*. v.21, n.w3, p. 389-401 2015.

74. Vigorito AC, Campregher P V., Storer BE, *et al.* Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. *Blood*. v. 114, n.3, p. 702-708, 2009.

75. Cuvelier GDE, Nemecek ER, Wahlstrom JT, *et al.* Benefits and challenges with diagnosing chronic and late acute GVHD in children using the NIH consensus criteria. *Blood*. v.134, n.3, p. 304-316, 2019.

76. Fainardi V, Lombardi E. Lung function tests to monitor respiratory disease in preschool children. *Acta Biomed.* v. 89, n. 2, p. 148-156, 2018.

77. Filipovich AH, Weisdorf D, Pavletic S, *et al.* National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. Diagnosis and Staging Working Group Report. Biol. *Blood Marrow Transplant*. v. 11, n. 12, p. 945-956, 2005.

78. Koc S, Leisenring W, Flowers MED, *et al.* Therapy for chronic graft-versus-host disease: a randomized trial comparing cyclosporine plus prednisone versus prednisone alone. *Blood*. v. 100, n.1, p. 48-51, 2002.

79. Flowers MED, Martin PJ. How we treat chronic graft-versus-host disease. *Blood*. v. 125, n. 4, p. 606-615, 2015.

80. Cutler C, Miklos D, Kim HT, *et al*. Rituximab for steroid-refractory chronic graft-versus-host disease. *Blood*. v. 108, n. 2, p. 756-762, 2006.

81. Dignan FL, Scarisbrick JJ, Cornish J, *et al.* Organ-specific management and supportive care in chronic graft-versus-host disease. *Br. J. Haematol.* v. 158, n.1, p. 62-78, 2012.

82. Greinix HT, van Besien K, Elmaagacli AH, *et al.* Progressive Improvement in Cutaneous and Extracutaneous Chronic Graft-versus-Host Disease after a 24-Week Course of Extracorporeal Photopheresis—Results of a Crossover Randomized Study. Biol. *Blood Marrow Transplant*. v. 17, n.1, p. 1775-1782, 2011.

83. Flowers MED, Apperley JF, van Besien K, *et al.* A multicenter prospective phase 2 randomized study of extracorporeal photopheresis for treatment of chronic graft-versus-host disease. *Blood*. v.112, n. 7, p. 2667-2674, 2008.

84. Greinix HT, Worel N, Just U, Knobler R. Extracorporeal photopheresis in acute and chronic graft-versus-host disease. *Transfus. Apher. Sci.* v.50, n. 3, p. 349-57, 2014.

85. Perotti C, Sniecinski I. A concise review on extracorporeal photochemotherapy: Where we began and where we are now and where are we going! *Transfus. Apher.* Sci. v. 52, n. 3, p. 360-368, 2015.

86. Martin PJ, Storer BE, Rowley SD, *et al.* Evaluation of mycophenolate mofetil for initial treatment of chronic graft-versus-host disease. *Blood*. v. 113, n.21, p. 5074-5082, 2009.

87. Lopez F, Parker P, Nademanee A, *et al.* Efficacy of mycophenolate mofetil in the treatment of chronic graft-versus-host disease. *Biol. Blood Marrow Transplant.* v. 11, n. 4, p. 307-313, 2005.

88. Zeiser R, Nguyen VH, Beilhack A, *et al.* Inhibition of CD4+CD25+ regulatory T-cell function by calcineurin-dependent interleukin-2 production. *Blood*. v. 108, n.1, p. 390-399, 2006.

89. Jurado M, Vallejo C, Pérez-Simón JA, *et al.* Sirolimus as Part of Immunosuppressive Therapy for Refractory Chronic Graft-versus-Host Disease. *Biol. Blood Marrow Transplant.* v. 13, n.6, p. 701-706, 2007.

90. Couriel DR, Saliba R, Escalon MP, *et al.* Sirolimus in combination with tacrolimus and corticosteroids for the treatment of resistant chronic graft-versus-host disease. *Br. J. Haematol.* v.130, 3, p. 409-417, 2005.

91. von Bonin M, Oelschlägel U, Radke J, *et al.* Treatment of Chronic Steroid-Refractory Graft-Versus-Host Disease With Low-Dose Rituximab. *Transplantation*. v.86, n. 6, p. 875-879, 2008.

92. Arai S, Pidala J, Pusic I, *et al*. A Randomized Phase II Crossover Study of Imatinib or Rituximab for Cutaneous Sclerosis after Hematopoietic Cell Transplantation. *Clin. Cancer Res.* v.22, n.2, p. 319-327, 2016.

93. Olivieri A, Locatelli F, Zecca M, *et al.* Imatinib for refractory chronic graft-versus-host disease with fibrotic features. *Blood*.v. 114, n. 3, p. 709-718, 2009.

94. Giaccone L, Martin P, Carpenter P, *et al.* Safety and potential efficacy of low-dose methotrexate for treatment of chronic graft-versus-host disease. *Bone Marrow Transplant.* v. 114, n.3, p. 337-41, 2005.

95. Inagaki J, Nagatoshi Y, Hatano M, *et al.* Low-dose MTX for the treatment of acute and chronic graft-versus-host disease in children. *Bone Marrow Transplant.* v.41, n.6, p. 571-577, 2008.

96. Miklos D, Cutler CS, Arora M, *et al.* Ibrutinib for chronic graft-versus-host disease after failure of prior therapy. v.130, n. 21, p. 2243-2250, 2017.

97. Jaglowski SM, Blazar BR. How ibrutinib, a B-cell malignancy drug, became an FDA-approved second-line therapy for steroid-resistant chronic GVHD. *Blood* Adv. v. 2, n.15, p. 2012-2019, 2018.

98. Escamilla Gómez V, García-Gutiérrez V, López Corral L, et al. Ruxolitinib in refractory acute and chronic graft-versus-host disease: a multicenter survey study. *Bone Marrow Transplant.* v.55, n. 3, 641-648, 2020.