Graft-Versus-Host Disease: Mechanism of Disease

MSL-DIS-US-0022 11/23

Presentation Overview

SECTION 1 — Graft-Versus-Host Disease (GVHD) Overview

SECTION 2 — Acute GVHD

SECTION 3 — Chronic GVHD

SECTION 4 — Appendix

Click the section tab of interest to be taken to the corresponding portion of the presentation

GVHD Overview

GVHD Results From Immunologic Attack on Recipients' Target Organs or Tissues by Donor Allogeneic T Cells[1]

aGVHD

- Activated donor T cells damage host epithelial cells and mucous membranes after an increase in inflammation following the conditioning regimen for HSCT[2,3]
- Organs affected: primarily skin, gut, and liver[3]

cGVHD

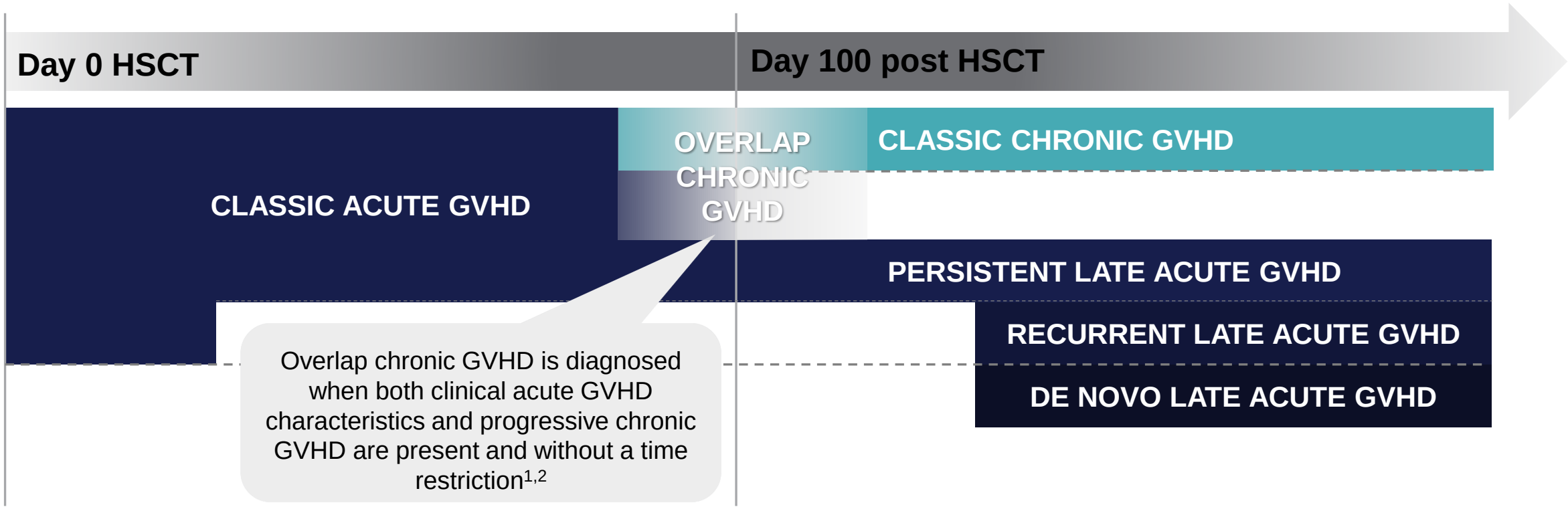
- Complex immune-mediated pathology of cGVHD involves T cells, B cells, macrophages, and fibroblasts[4,5]
- Displays more autoimmune and fibrotic features than aGVHD[4,5]
- Organs affected: primarily oral and ocular mucosal surfaces; may also affect the skin, gut, liver, lungs, and kidneys[2,5]

- Loss of central tolerance, autoantibody production, and fibrosis in cGVHD are thought to distinguish cGVHD from aGVHD[5,6]
- "Overlap cGVHD" is characterized by clinical features of both aGVHD and cGVHD[5,7]

aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation.

1. Blazar BR, et al. *Nat Rev Immunol*. 2012;12:443-458. 2. Jacobsohn DA, Vogelsang GB. *Orphanet J Rare Dis*. 2007;2:35. 3. Vogelsang GB, et al. *Annu Rev Med.* 2003;54:29-52. 4. McDonald-Hyman C, et al. *Sci Transl Med.* 2015;7:280rv2. 5. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 6. Abboud R, et al. *Ther Adv Hematol*. 2020;11: 1-13. 7. Lee SJ. *Blood*. 2017;129:30-37.

Classification of GVHD



Acute and chronic GVHD are considered distinct clinical syndromes without a time restriction[1]

Figure adapted from Lee SJ. *Blood*. 2017;129:30-37.[1]
Note: Box sizes do not reflect relative prevalence or incidence.

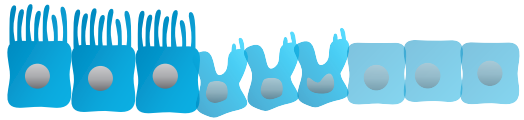
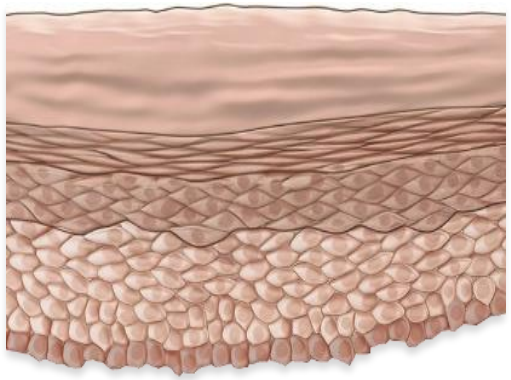
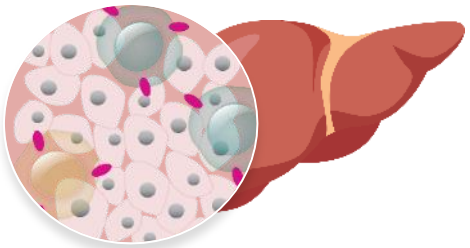
1. Lee SJ. *Blood*. 2017;129:30-37. 2. Filipovich AH, et al. *Biol Blood Marrow Transplant*. 2005;11:945-956.

Acute GVHD

Mechanism of Disease

Clinical Manifestations of aGVHD

- In aGVHD, the conditioning regimen causes tissue damage, which activates T cells[1]
- Activated T cells expand and differentiate into cytotoxic effector T cells[1]
- Effector T cells migrate from lymphoid tissues to cause organ damage[1]

	Gut	Skin	Liver
			
	Colonic mucosa may develop withered and necrotic crypts. Large deep ulcers are seen with mucosal sloughing and loss of epithelium[2]	Cutaneous manifestations in aGVHD can affect the skin causing erythematous maculopapular rashes on the face, ears, palms and soles[1]	Bile duct damage may occur in hepatic GVHD. The epithelial cells of the bile duct may show eosinophilic cytoplasm and variable nuclear hyperchromasia with crowding[3]
Most common symptoms[4]	Diarrhea	Rash	Hyperbilirubinemia

1. Rodrigues KS, et al. *Am J Clin Dermatol.* 2018;e19:33-50. 2. Naymagon S, et al. *Nat Rev Gastroenterol Hepatol.* 2017;14:711-726. 3. Stueck AE, et al. *Mod Pathol.* 2018;31:442-451. 4. Ferrara JLM, et al. *Lancet*. 2009;373:1550-1561.

aGVHD: Epidemiology and Significance

Serious complications of allo-HSCT with significant morbidity and mortality[1,2]

- Incidence of acute GVHD varies by donor type and prophylaxis regimen
  - MSD: approximately 30% to 40%[3]
  - MUD or mismatched relative: approximately 20% to 50%[4-5]
  - Standard prophylaxis (CNI+MTX/MFF±ATG): ranges from 30% to 60%[6,7]
- In recent studies, death due to complications of aGVHD was reported in 16% to >30% of patients[8-10]

Immunosuppression with corticosteroids is first-line therapy but is insufficient for most patients[11,12]

- Less than 50% of patients with aGVHD experience clinically relevant responses to first-line therapy[11]

May rapidly progress to steroid-refractory disease[13]

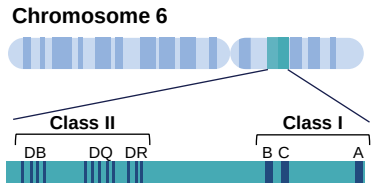
- Steroid-refractory aGVHD has been reported to have a mortality rate of approximately 35%[9]

allo-HSCT, allogeneic hematopoietic stem cell transplantation; ATG, antithymocyte globulin; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; MSD, matched sibling donor; MTX, methotrexate; MUD, matched unrelated donor.

1. Jagasia M, et al. *Blood*. 2012;119:296-307. 2. Jacobsohn DA, Vogelsang GB. *Orphanet J Rare Dis*. 2007;2:35. 3. Nagler A, et al. *Transplant Cell Ther*. 2022;28(2):86.e1-86.e8. 4. Nagler A, et al. *Clin Cancer Res.* 2021;27(3):843-851. 5. Luznik L, at al. *J Clin Oncol.* 2022;40(4):356-368. 6. Jamy O, et al. *Blood*. 2023;142(12):1037-1046. 7. Malard F, et al. *Nat Rev Dis Primers*. 2023;9(1):27. 7. Yu J, et al. *Curr Med Res Opin*. 2019;35:983-988. 8. Yu J, et al. *Biol Blood Marrow Transplant.* 2020;26:600-605. 9. Ramdial JL, et al. *Bone Marrow Transplant.* 2021;56:2005-2012. 10. Garnett C, et al. *Ther Adv Hematol*. 2013;4:366-378. 11. Magenau J, et al. *Br J Haematol*. 2016;173:190-205. 12. Schoemans HM, et al. *Bone Marrow Transplant.* 2018;53:1401-1415.

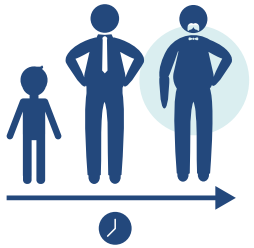
Risk Factors for Development of aGVHD

HLA[1-3]



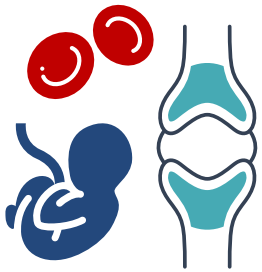
Risk of GVHD is impacted by HLA

Age[4-6]



Older age of donor and recipient (≥40 years) has been associated with higher risk of aGVHD

Graft type[5,6]



Source of stem cells also has an impact on aGVHD risk (ie, peripheral blood > bone marrow > umbilical cord)

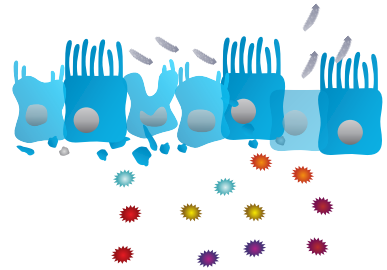
Conditioning regimens[1,7]

Chemotherapy

Radiation

Prior to transplant, the conditioning phase can lead to activation of innate immune cells that participate in direct tissue damage

Changes in gut microbiome[5,8-10]

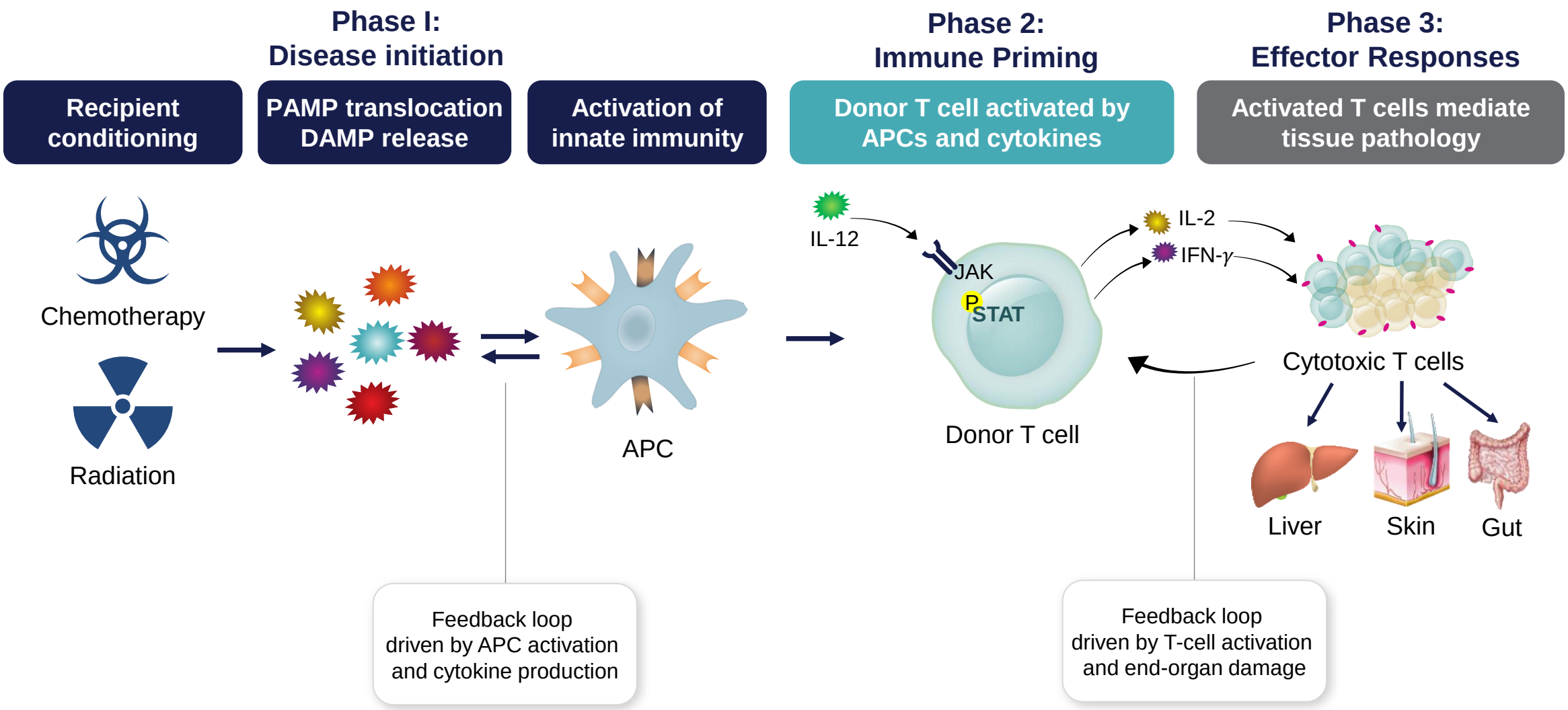


Damage to the GI tract can result in the release of microbial products into systemic circulation, stimulating cytokine production

GI, gastrointestinal; HLA, human leukocyte antigen.

1. Ferrara JLM, et al. *Lancet*. 2009;373:1550-1561. 2. Loiseau P, et al. *Biol Blood Marrow Transplant.* 2007;13:965-974. 3. Flowers MED, et al. *Blood.* 2011;117:3214-3219. 4. Nash RA, et al. *Blood.* 1992;80:1838-1845. 5. Nassereddine S, et al. *Anticancer Res*. 2017;37:1547-1555. 6. Cutler C. *Hematology.* 2008;3(part 1):1-12. 7. Blazar BR, et al. *Nat Rev Immunol.* 2012;12:443-458. 8. Whangbo J, et al. *Bone Marrow Transplant.* 2017;52:183-190. 9. Taur Y, et al. *Blood.* 2014;124;1174-1182. 10. Jenq RR, et al. *J Exp Med*. 2012;209:903-911.

Overview of aGVHD Pathophysiology



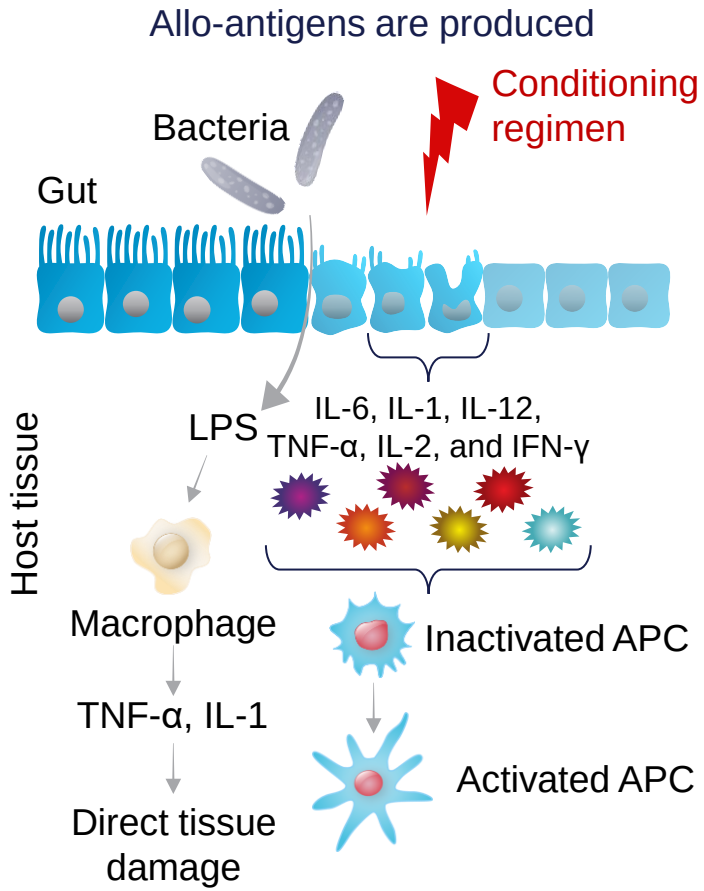
APC, antigen presenting cell; DAMP, damage-associated molecular pattern; IFN, interferon; IL, interleukin; JAK, Janus kinase; P, phosphorylated; PAMP, pathogen-associated molecular pattern; STAT, signal transducer and activator of transcription.

Hill GR, et al. *Ann Rev Immunol*. 2021;39:19-49.

Disease Initiation: Damage to Host Tissue Activates Donor and Host APCs

- Occurs due to HLA mismatch, conditioning, and/or other factors[1,2]
- Conditioning enables host recipients to receive immunocompetent T cells[3,4]
- Conditioning-mediated tissue injury to host mucosa, skin, and liver leads to cytokine release and innate immune activation, initiating GVHD[3,5-8]
- Activated donor and host APCs then release their own cytokines, resulting in a positive feedback loop[2,4]

Disease initiation caused by recipient conditioning and other factors[1,8-10]



LPS, lipopolysaccharide; TNF, tumor necrosis factor.

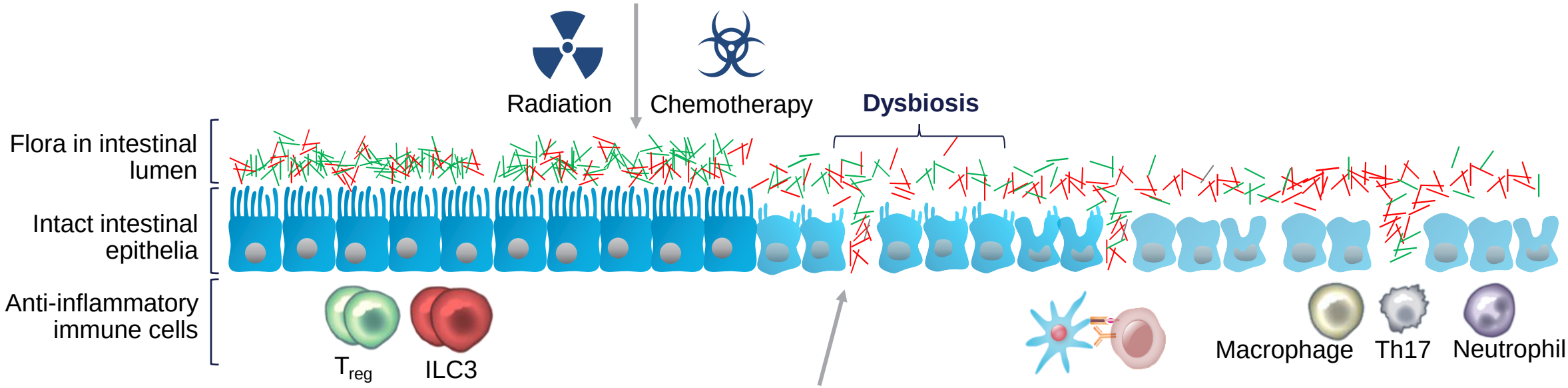
1. Nassereddine S, et al. *Anticancer Res.* 2017;37:1547-1555. 2. McDonald-Hyman C, et al. *Sci Transl Med.* 2015;7:280-282. 3. Ferrara JL, et al. *Stem Cells.* 1996;14:473-489. 4. Blazar BR, et al. *Nat Rev Immunol.* 2012;12:443-458. 5. Toubai T, et al. *Front Immunol.* 2016;7:539. 6. Markey KA, et al. *Blood*. 2014;124:354-362. 7. Zeiser R, Blazar BR. *N Engl J Med.* 2017;377:2167-2179. 8. Abboud R, et al. *Ther Adv Hematol*. 2020;11:1-13. 9. Schroeder MA, DiPersio JF. *Dis Model Mech*. 2011;4:318-333. 10. Ferrara JLM, et al. *Lancet*. 2009;373:1550.

Disease Initiation: Changes in Gut Microbiome Contribute to aGVHD

Normal gut barrier[1,2]

Conditioning regimen

Postconditioning regimen[1]



Bacterial translocation leads to the activation of APCs in a process that involves STAT signaling[3]

APCs recognize pathogenic bacteria and prime donor T cells[2]

JAK/STAT and PI3K signaling in macrophages causes damage to target tissues via nitric oxide production[3]

ILC, innate lymphoid cell; PI3K, phosphoinositide 3 kinase; Th, T helper; T_{reg}, regulatory T cell.

1. Rafei H, Jenq RR. *Blood*. 2020;136:401-409. 2. Chen Y, et al. *J Immunol Res*. 2015;2015:145859. 3. Abboud R, et al. *Ther Adv Hematol*. 2020;11:2040620720914489.

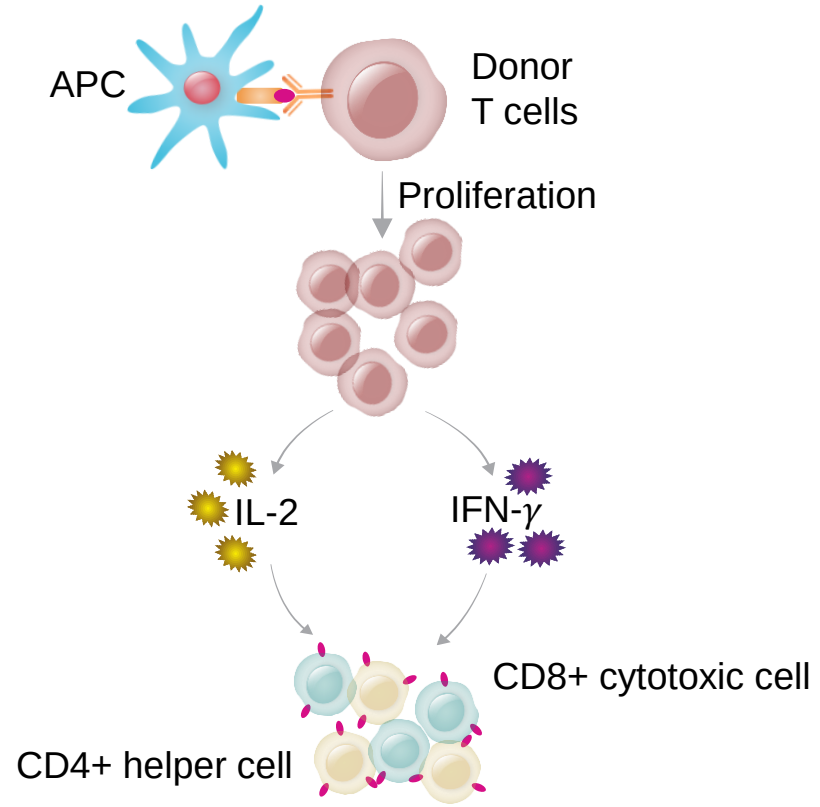
Donor T-Cell Activation and Expansion

2
Immune priming

- T-cell receptors and co-stimulatory molecules (eg, CD28) on donor T cells interact with ligands on the surface of the activated APCs, leading to donor T-cell activation[1]
- Inflammatory cytokines produced in response to these activation signals subsequently stimulate T-cell expansion and differentiation[1,2]
- Signaling through the IFN-ɣ receptor and the JAK/STAT pathway results in increased T-cell trafficking to the gut, liver, and skin[2]

Donor T-cell activation[2-4]

Production of cytolytic donor effector T cells



CD, cluster of differentiation.

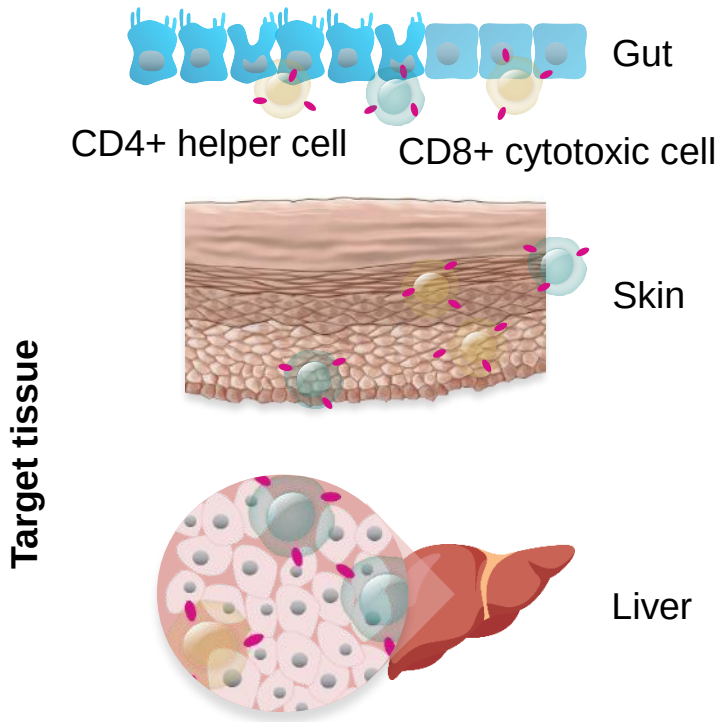
1. Villa NY, et al. *Viruses*. 2016;8:1-30. 2. Abboud R, et al. *Ther Adv Hematol*. 2020;11:1-13. 3. Nassereddine S, et al. *Anticancer Res*. 2017;37:1547-1555. 4. McDonald-Hyman C, et al. *Sci Transl Med*. 2015;7:280-282.

End-Organ Damage Potentiates aGVHD

- Donor T-cell trafficking to target organs is mediated by chemokines, chemokine receptors, and adhesion molecules[1-3]
- Recruited T cells from lymphoid tissues[4-6]
  - Proliferate and differentiate into effector T cells
  - Migrate to target organs
  - Release additional cytokines and other immune effectors to cause inflammation, organ damage, and apoptosis
- Sustained end-organ tissue damage potentiates aGVHD through cytokine-mediated feedback loops and maintenance of inflammation[2]

End-organ damage[4,7]

Cytolytic donor cells cause end-organ damage and perpetuate pathway propagation



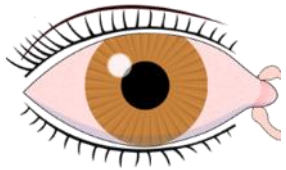
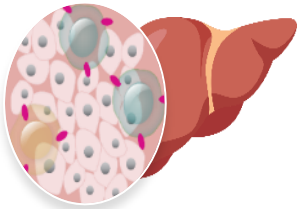
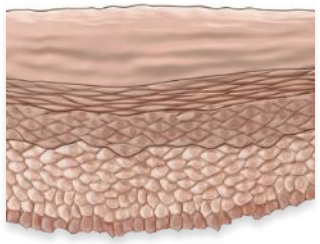
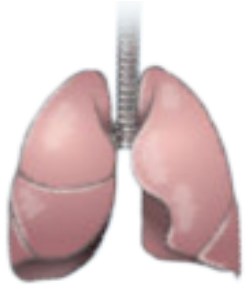
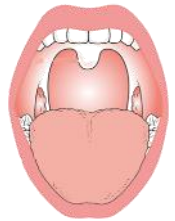
1. Ferrara JLM, et al. *Lancet*. 2009;373:1550-1561. 2. McDonald-Hyman C, et al. *Sci Transl Med*. 2015;7:280-282. 3. Schroeder MA, et al. *Biol Blood Marrow Transplant.* 2018;24:1125-1134. 4. Villa NY, et al. *Viruses*. 2016;8:1-30. 5. Blazar BR, et al. *Nat Rev Immunol.* 2012;12:443-458. 6. Ball LM, et al. *Bone Marrow Transplant.* 2008;41(suppl 2):S58-S64. 7. Nassereddine S, et al. *Anticancer Res*. 2017;37:1547-1555.

Chronic GVHD

Mechanism of Disease

Clinical Manifestations of cGVHD

- cGVHD is the most common long-term complication of allo-HSCT[1]
- Among patients receiving allo-HSCT, 30% to 50% experience cGVHD[2-5]
- cGVHD has a median onset of 4 to 6 months after allo-HSCT[6,7]

	Eyes	Liver	Skin	Lung	Mouth
					
	cGVHD of the eye can have characteristic hypervasculature[8]	Inflammatory cellular infiltrates may occur in the bile duct of a patient with cGVHD[8]	Localized sclerosis of the skin occurs in cGVHD[8]	Pronounced fibrotic changes can be seen in the lungs of a patient with cGVHD[8]	Oral cGVHD is characterized by reduced and sclerotic gingiva[8]
Common symptoms[9]	Dry eyes	Jaundice	Dyspigmentation	Bronchiolitis	Dry mouth

1. Lee SJ. *Blood.* 2017;129:30-37. 2. Kitco CL, et al. *Transplant Cell Ther.* 2021;27(7):545-557. 3. Im A, et al. *Biol Blood Marrow Transplant.* 2020;26(8):1459-1468. 4. Arai S, et al. *Biol Blood Marrow Transplant.* 2015;21(2):266-274. 5. Arora M, et al. *Biol Blood Marrow Transplant.* 2016;22(3):449-455. 6. Lee SJ. *Best Pract Res Clin Haematol.* 2010;23:529-535. 7. Garnett C, et al. *Ther Adv Hematol.* 2013;4:366-378. 8. Zeiser R, Blazer BR. *N Engl J Med.* 2017;377:2565-2579. 9. Ferrara JLM, et al. *Lancet*. 2009;373:1550.

cGVHD: Epidemiology and Significance

cGVHD is associated with significant morbidity[1-4]

- Many patients require ongoing immunosuppressive therapy years after diagnosis of cGVHD
  - A large proportion of patients may need immunosuppressive therapy years after diagnosis depending on severity of disease[5,6]
- cGVHD is associated with frequent and severe infections[2,4,7]
- cGVHD leads to debilitating fibrotic organ damage that can be irreversible[8]

cGVHD is associated with worse patient-reported outcomes compared with healthy individuals, including[1,9-11]

- Significantly lower health-related quality of life
- Decreased functional status
- Inability to work or resume social roles

Among patients who are disease-free after allo-HSCT, cGVHD is a leading cause of NRM[1,12,13]

- 60% to 80% 2-year OS and RFS rates[3,a]
- cGVHD-associated NRM increases over time and is associated with organ failure and infection[13]

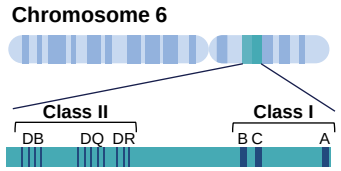
[a] Two-year survival outcomes are based on data from the Fred Hutchinson Cancer Research Center.[3]

NRM, non-relapse mortality; OS, overall survival; RFS, relapse-free survival.

1. Lee SJ. *Blood*. 2017;129:30-37. 2. Blazar BR, et al. *Nat Rev Immunol*. 2012;12:443-458. 3. Lee SJ. *Best Pract Res Clin Haematol.* 2010;23:529-535. 4. Garnett C, et al. *Ther Adv Hematol*. 2013;4:366-378. 5. Stewart BL, et al. *Blood.* 2004;104:3501-3506. 6. Curtis LM, et al. *Biol Blood Marrow Transplant.* 2017;23:1980-1988. 7. Socie G, Ritz J. *Blood.* 2014;124:374-384. 8. Hill GR, et al. *Annu Rev Immunol*. 2021 Apr 26;39:19-49. 9. Pidala J, et al. *Blood*. 2011;117:4651-4657. 10. Lee SJ, et al. *Haematologica*. 2018;103:1535-1541. 11. Kurosawa S, et al. *Biol Blood Marrow Transplant*. 2019;25:1851-1858. 12. Wingard JR, et al. *J Clin Oncol*. 2011;29:2230-2239. 13. DeFilipp Z, et al. *Blood Adv*. 2021;5(20):4278-4284.

Risk Factors for Development of cGVHD

HLA mismatch[1-5]



HLA mismatch between donor and recipient is a significant risk factor influencing the development of cGVHD

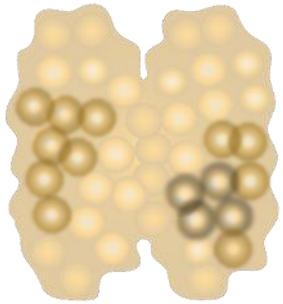
Conditioning regimens[1-3]

Chemotherapy

Radiation

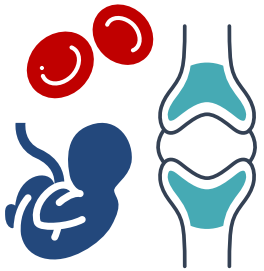
Higher intensity conditioning causes more tissue damage, enhancing cytokine release and immune activation

Prior aGVHD[1,3-5]



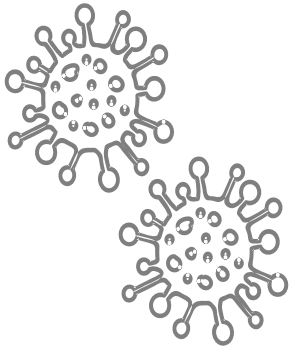
Prior tissue damage from aGVHD, especially to the thymus, may persist and contribute to cGVHD initiation

Graft characteristics[1-5]



PBSCT is associated with greater risk of cGVHD compared with BMT or UCBT

Prior EBV infection[1-3]



Seropositivity of herpes viruses, including EBV, suggests a mechanism that could exacerbate or initiate cGVHD

Post-HSCT treatments[1-5]

Treatments involving T-cell depletion or post transplantation cyclophosphamide may **decrease** incidence and severity of cGVHD

BMT, bone marrow transplant; EBV, Epstein-Barr virus; PBSCT, peripheral blood stem cell transplant; UCBT, umbilical cord blood transplant.

1. Zeiser R, Blazar BR. *N Engl J Med.* 2017;377:2565-2579. 2. Styczynski J, et al. *J Clin Oncol.* 2016;34:2212-2220. 3. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 4. Lee SJ. *Blood*. 2017;129:30-37. 5. Filipovich AH, et al. *Biol Blood Marrow Transplant*. 2005;11:945-956.

Review of cGVHD Pathophysiology

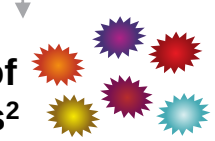
Disease initiation[1]

Chemotherapy

Radiation

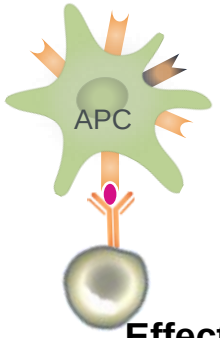
Host Tissue Damage

Release of Cytokines[2]

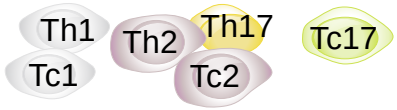


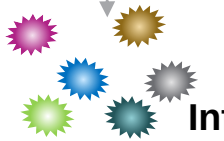
T-cell phase[3]

T-cell Activation



Effector T-Cell Differentiation

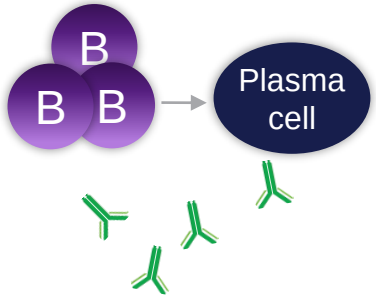




Chronic Inflammation

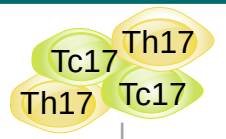
B-cell Phase[2,3]

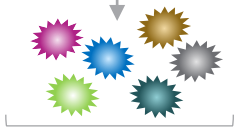
Aberrant B-cell activation/expansion



Constitutive allo/autoantibody production

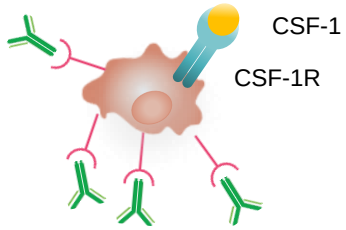
Macrophage/fibrosis phase[3]



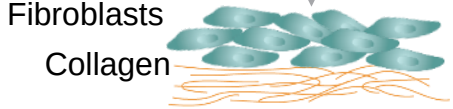


CSF-1, IFN-γ, IL-22, TNF, IL-13, IL-17

Macrophage Survival and Activation



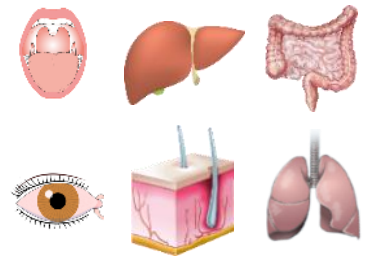
TGF-β



End-organ damage[2,3]

Fibroblast Proliferation

cGVHD Target Tissues



End-Organ Damage

BAFF, B cell activating factor; CSF, colony-stimulating factor; Tc, cytotoxic T cell; TGF-β, transforming growth factor beta.

1. Toubai T, et al. *Front Immunol.* 2016;7:539. 2. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 3. MacDonald KPA, et al. *Blood*. 2017;129:13-21.

Disease Initiation: Damage to the Host Tissue

1 Response to conditioning regimen–induced tissue damage

Conditioning enables host recipients to receive immunocompetent T cells[1,2]

Radiation

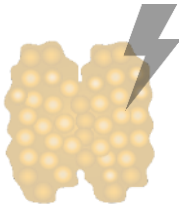
Chemotherapy

Damage

Conditioning regimens may comprise a combination of chemotherapy, radiation, and/or immunosuppressive drugs

2 Host tissue damage

Damage to the thymus from the conditioning regimen or other causes[a] results in impaired negative selection, leading to the development of host-derived donor reactive T cells[2-4]

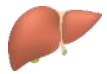


Thymus

Conditioning-mediated damage to host mucosa and skin initiates GVHD[5]

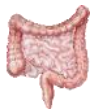
Mouth

Eyes

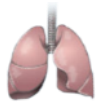


Liver

Skin



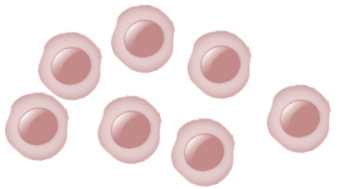
Gut



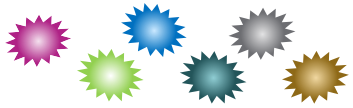
Lungs

3 Cytokine release and acute inflammation

Donor reactive T cells



Release of cytokines



Damage to host tissues causes release of cytokines, leading to[3,5]

- Donor T-cell differentiation into Th1/Tc1, Th2/Tc2, and Th17 effector cells[6,7]
- Recruitment of donor T cells into the target tissue[6,7]

[a] Other causes of thymic injury include prophylaxis with calcineurin inhibitors, alloreactive T cells, low serum thymic hormone levels, and immunoglobulin deposition.[3]

1. Ferrara JL, et al. *Stem Cells.*1996;14:473-489. 2. Blazar BR, et al. *Nat Rev Immunol.* 2012;12:443-458. 3. Zeiser R, Blazar BR. *N Engl J Med*. 2017;377:2565-2579. 4. Soares MV, et al. *Front Immunol*. 2019;10:334. 5. Toubai T, et al. *Front Immunol.* 2016;7:539. 6. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 7. Schroeder MA, DiPersio JF. *Dis Model Mech*. 2011;4:318-333.

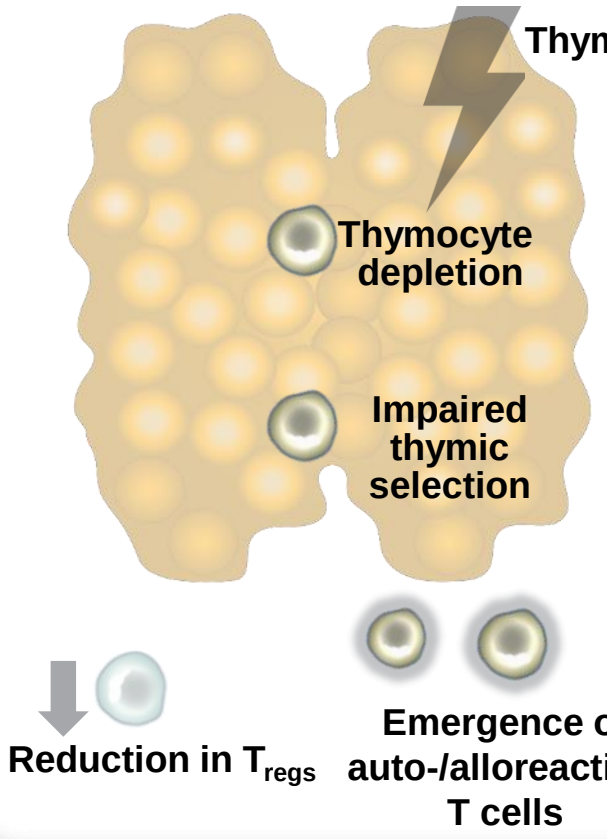
Dysregulated T-Cell Immunity: Thymic Dysfunction Contributes to cGVHD

2

T-cell phase

- Thymic injury, caused by the conditioning regimen, alloreactive T cells, or prior aGVHD, leads to[1-6]
  - Emergence of alloreactive and autoreactive T cells due to **impaired thymic selection**
  - **Thymocyte depletion** resulting in the loss of T_{regs}, further facilitating escape of auto- and alloreactive T cells into the periphery
- Disrupted immune regulation leads to activation of autoreactive and alloreactive T cells, further propagating cGVHD pathology[2,5-7]

Central and Peripheral Tolerance Mechanisms[7]

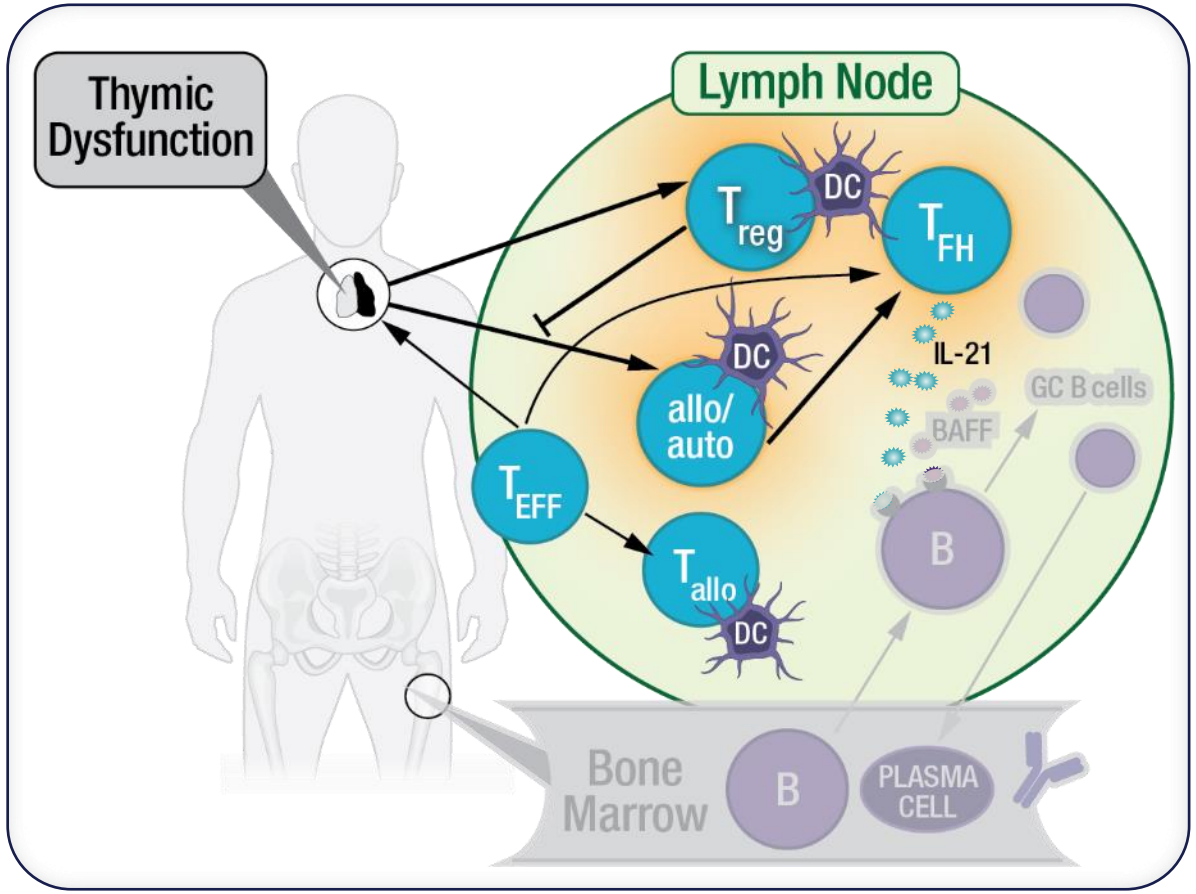


1. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 2. MacDonald KPA, et al. *Blood*. 2017;129:13-21. 3. Wu T, et al. *J Immunol*. 2013;191:488-499. 4. Zeiser R, Blazar BR. *N Engl J Med*. 2017;377:2565-2579. 5. McDonald-Hymen C, et al. *Sci Transl Med*. 2015;7:280-282. 6. Beres AJ, Drobyski WR. *Front Immunol*. 2013;4:1-9. 7. Gregersen PK, Behrens TW. *Nat Rev Genet*. 2006;7:917-928.

Dysregulated T-Cell Immunity: An Imbalance Between Effector Cells and T_{regs}

2

T-cell phase



- Dysregulation of immune mechanisms via[1-5]
  - Donor T-cell activation
  - Autoreactive T-cell proliferation
  - Reduced T_{regs}
- Activation of donor lymphocytes leads to differentiation into effector populations[1,3,5]
- Cytolytic attack by effector T cells leads to further recruitment of immune cells[1]
- Proinflammatory cytokines and an imbalance in effector and regulatory populations promote chronic inflammation, leading to widespread tissue fibrosis and exacerbating cGVHD[1-7]

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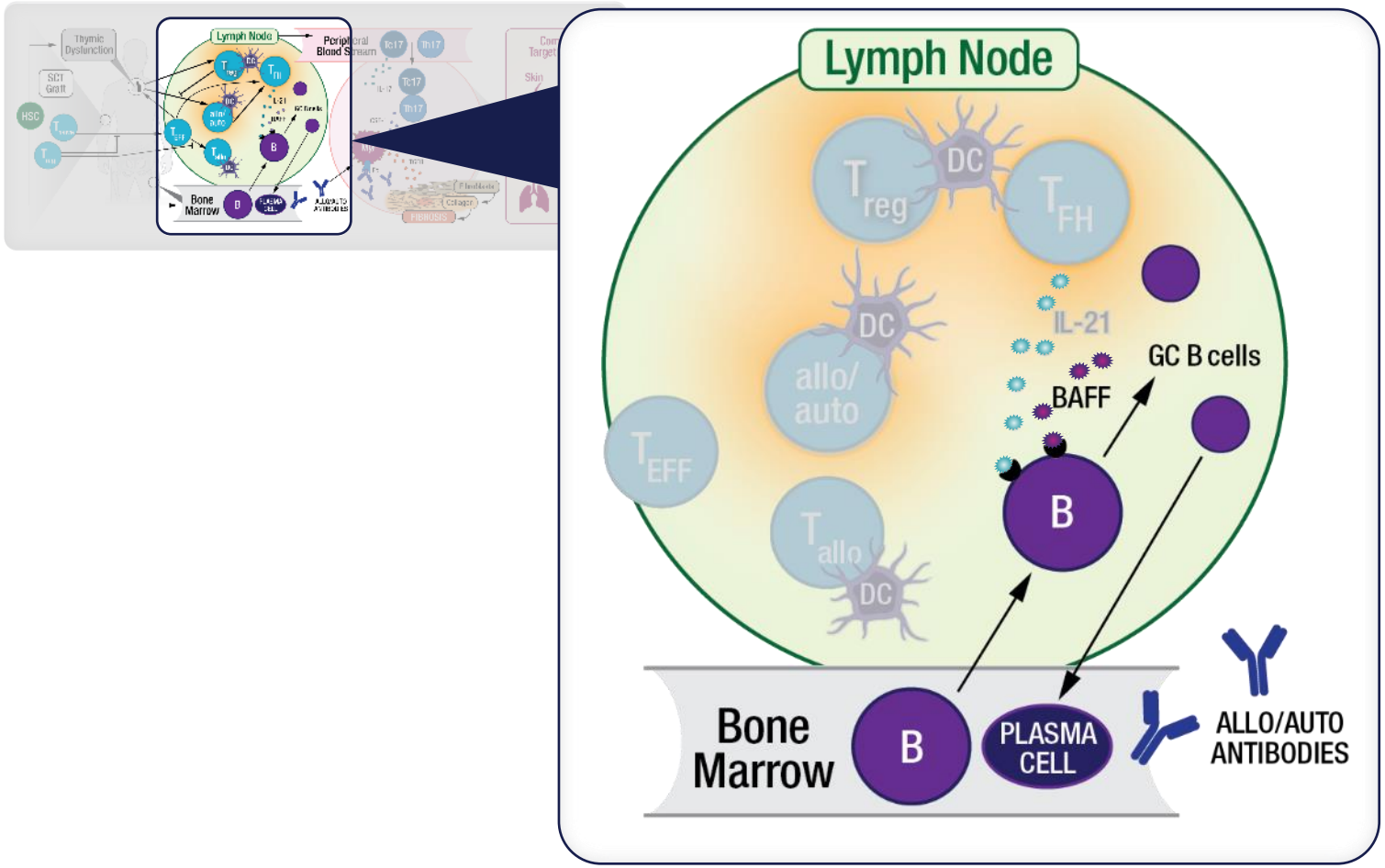
DC, dendritic cell; T_{EFF}. effector T cell; T_{FH}, T follicular helper cell.

1. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 2. Blazar BR, et al. *Nat Rev Immunol.* 2012;12:443-458. 3. Zeiser R, Blazar BR. *N Engl J Med*. 2017;377:2565-2579. 4. MacDonald KPA, et al. *Blood*. 2017;129:13-21. 5. Schroeder MA, DiPersio JF. *Dis Model Mech*. 2011;4:318-333. 6. MacDonald KPA, et al. *J Clin Invest.* 2017;127:2452-2463. 7. McDonald-Hymen C, et al. *Sci Transl Med*. 2015;7:280-282.

Aberrant B-Cell Activation and Expansion

3

B-cell phase



- Aberrant B-cell activation and expansion occurs due to
  - Impaired B-cell reconstitution after HSCT[1-3]
  - Elevated expression of BAFF[1,3]
  - Interactions with donor T cells[3,4]
- Activated B cells capable of constitutive **allo/autoantibody production** contribute to the inflammatory response and target tissue damage[1-7]

Reproduced from *Blood*, Vol 129, MacDonald KPA, et al. Chronic graft-versus-host disease: biological insights from preclinical and clinical studies, Pages 13-21, Copyright (2017), with permission from Elsevier.
GC, germinal center.

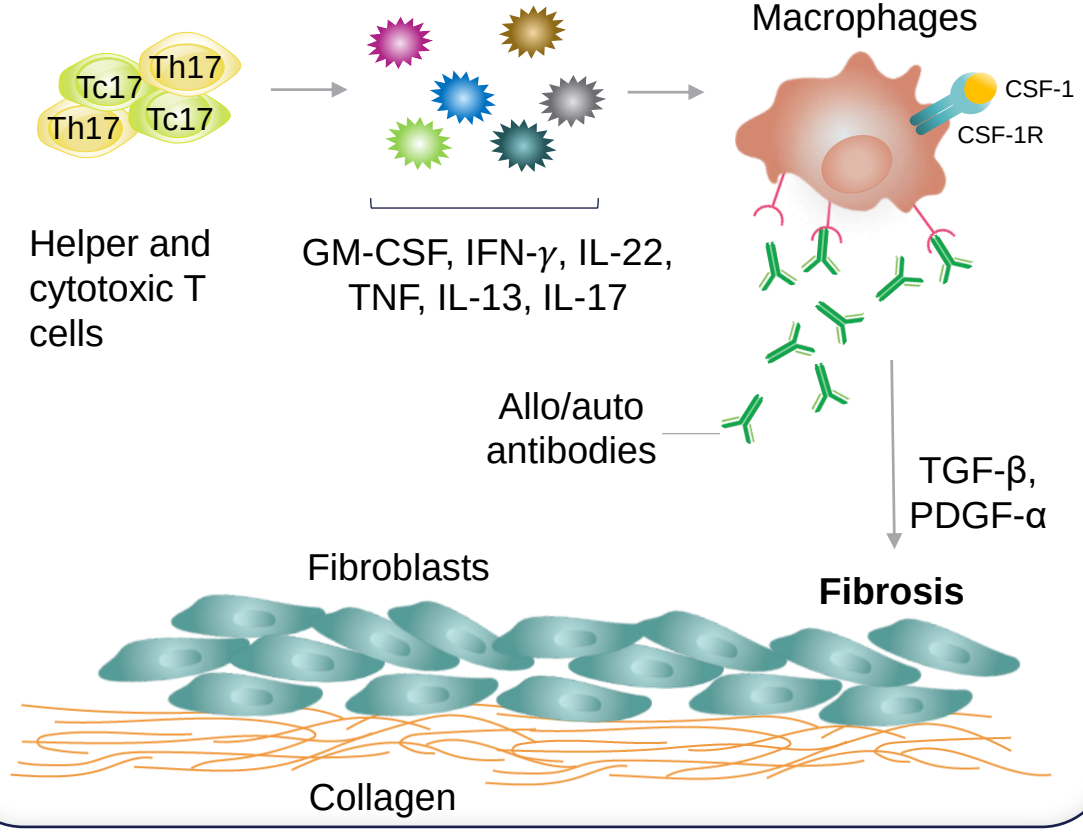
1. Sarantopoulos S, et al. *Biol Blood Marrow Transplant*. 2015;21:16-23. 2. Shimabukuro-Vornhagen A, et al. *Blood*. 2009;114:4919-4927. 3. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 4. Schroeder MA, DiPersio JF. *Dis Model Mech*. 2011;4:318-333. 5. MacDonald KPA, et al. *Blood*. 2017;129:13-21. 6. Zeiser R, Blazar BR. *N Engl J Med.* 2017;377:2565-2579. 7. McDonald-Hyman C, et al. *Sci Transl Med*. 2015;7:280rv2.

4
Macrophage / Fibrosis phase

Activated Macrophages Produce Inflammatory Cytokines and Growth Factors That Drive Fibrosis

- Effector cell–produced proinflammatory cytokines and B-cell–derived allo/autoantibodies promote macrophage activation[1-4]
- Donor-derived macrophages are dependent on CSF-1R signaling for proliferation, differentiation, and migration[5-11]
- CSF-1R–dependent activated macrophages mediate production of TGF-β, which contributes to intestinal pathology, epidermal inflammation, and subcutaneous and cutaneous fibrosis in cGVHD[12-13]

Fibroblast activation and collagen deposition[2]



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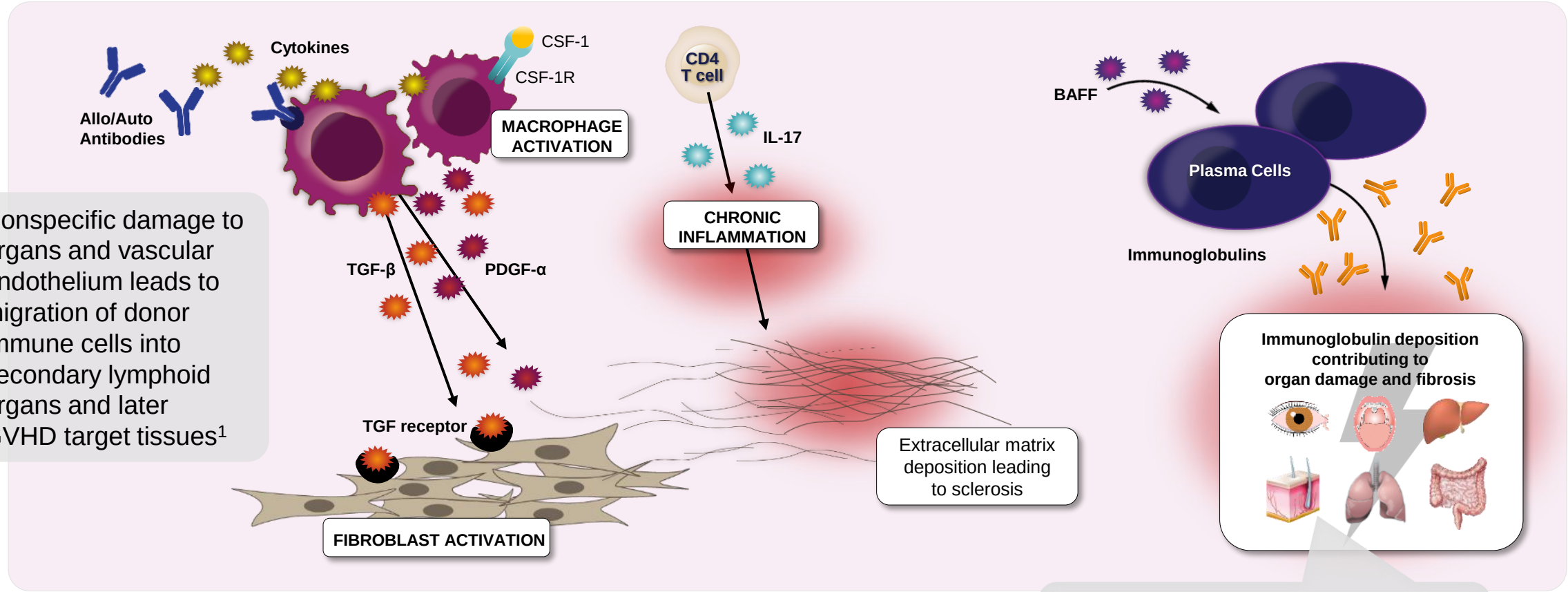
GM-CSF, granulocyte-macrophage colony-stimulating factor; PDGF-α, platelet-derived growth factor alpha.

1. MacDonald KPA, et al. *Blood*. 2017;129:13-21. 2. MacDonald KPA, et al. *J Clin Invest*. 2017;127:2452-2463. 3. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 4. Schroeder MA, DiPersio JF. *Dis Model Mech*. 2011;4:318-333. 5. Hume DA, et al. *J Immunol*. 1988;141(10):3405-3409. 6. Hume DA and MacDonald KP. *Blood*. 2012;119(8):1810-1820. 7. Kelley TW, et al. *J Biol Chem*. 1999;274(37):26393-26398. 8. Mossadegh-Keller N, et al. *Nature*. 2013;497(7448):239-243. 9. Rieger MA, et al. *Science*. 2009;325(5937):217-218. 10. Stanley ER and Chitu V. *Cold Spring Harb Perspect Biol*. 2014;6(6):a021857. 11. Tushinski RJ and Stanley ER. *J Cell Physiol*. 1983;116(1):67-75. 12. Alexander KA, et al. *J Clin Invest*. 2014;124(10):4266-4280. 13. Banovic T, et al. *Blood*. 2005;106(6):2206-2214.

Aberrant Tissue Repair, Propagation of Fibrosis, and Progression of cGVHD[1]

End-organ damage



Nonspecific damage to organs and vascular endothelium leads to migration of donor immune cells into secondary lymphoid organs and later GVHD target tissues[1]

Fibrosis of the skin and lungs can result in scleroderma-like changes and BOS, respectively[1-3]

Image adapted from Zeiser R, Blazar BR. *N Engl J Med*. 2017;377:2565-2579.
BOS, bronchiolitis obliterans syndrome.
1. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 2. MacDonald KPA, et al. *J Clin Oncol*. 2017;127:2452-2463. 3. Kitko CL, et al. *Biol Blood Marrow Transplant.* 2012;18:S46-S52.

Appendix

Histologic Features of cGVHD

- Displays autoimmune and fibrotic features[1]
- Inflammatory proteins and fibrotic changes cause diffuse, nonspecific damage to numerous organs and the vascular endothelium[1,2]

End Organ/Site	Characteristic Histopathologic Findings[3]
Liver	• Immune-mediated damage to small bile ducts and ductules • Cholestatic and inflammatory changes
Skin	• Superficial interface dermatitis with vacuolar change in the basilar layer • Lichenoid pattern of lymphocytic inflammation ± lymphocyte satellitosis
GI	• Destruction of basilar glands or crypts • Mucosal denudation • Enterocyte apoptosis
Mucosa (eg, oral cavity, eye)	• Exocytosis, apoptosis, and/or lichenoid interface inflammation characteristic of localized or generalized epithelial changes • Conjunctival features include lymphocyte exocytosis, satellitosis, vacuolization of the basal epithelium, and epithelial cell necrosis
Lungs	• Constrictive bronchiolitis obliterans (cGVHD) • Cryptogenic organizing pneumonia (aGVHD and cGVHD)

Note: Diagnosis of GVHD is not achieved by histopathology alone; histology results must be integrated into the context of clinical presentation.[2]
GI, gastrointestinal.
1. Blazar BR, et al. *Nat Rev Immunol.* 2012;12:443-458. 2. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 3. Shulman HM, et al. *Biol Blood Marrow Transplant*. 2015;21:589-603.

Potential Biomarkers for cGVHD

Biomarker	Biologic Function	Prognostic	Diagnostic	Predictive
BAFF	BAFF is a survival factor for B cells and controls normal B-cell maturation; however, it promotes survival of autoreactive B cells[1,2]; increased levels of sBAFF correlate with onset of, and active cGVHD. Increased levels 1 month after ECP predicted response of skin cGVHD[3,4]		✓	✓
B cells	A breakdown in peripheral B-cell tolerance and altered B-cell homeostasis are components of cGVHD[1]; an imbalance of certain B-cell subsets is associated with the diagnosis and severity of cGVHD[4,5]	✓	✓	✓
CD163	CD163 is a macrophage scavenger receptor regulated by inflammatory mediators[6]; plasma CD163 associates with de novo–onset cGVHD[3,4]	✓		
CTLA-4 SNP	Association between position +49 guanine to guanine homozygote genotype in donors and higher risk of cGVHD[5,7]	✓		
CXCL9	T cell type 1 chemokine detected in the blood that attracts CXCR3+ T cells in cGVHD target organs[8]; increased levels and up-regulated gene expression detected at diagnosis of cGVHD; increased levels at symptom onset associated with severe cGVHD[3-5]	✓	✓	
CXCL10	Increased levels and up-regulated gene expression detected at diagnosis and in active cGVHD[3-5]	✓	✓	
DKK3	Glycoprotein that regulates Wnt signaling, RYK and Ror2[8]; increased levels at diagnosis associated with NRM[3,4]	✓	✓	
IL-15	Homeostatic cytokine[5]; low levels of IL-15 on day 7 after allo-HSCT associated with a 2.7-fold higher likelihood of developing cGVHD[4,9]	✓	✓	
IL-2Rα	Decrease in sIL-2Rα predicts response to the combination of ECP and ruxolitinib[5]			✓
miRNAs	Play a regulatory role in immune response and autoimmunity; miR-155 strongly associated with onset and severity of cGVHD, and differentially expressed in patients with severe and mild cGVHD[5]		✓	

CTLA, cytotoxic T-lymphocyte antigen; CXCL, C-X-C Motif Chemokine Ligand; DKK, dickkopf; ECP, extracorporeal photophoresis; miRNAs, micro RNAs, NRM, nonrelapse mortality; Ror, receptor tyrosine kinase-like orphan receptor; RYK, receptor-like tyrosine kinase; sBAFF, soluble B-cell activating factor; SNP, single-nucleotide polymorphism; TRM, treatment-related mortality.

1. Sarantopoulos S, et al. *Blood*. 2009;113:3865-3874. 2. Liu Z, Davidson A. *Trends Immunol*. 2011;32:388-394. 3. Bidgoli A, et al. *Transpl Cel Ther.* 2022;28:657-666. 4. Milosevic E, et al. *Front Immunol.* 2022;13.1033263. doi: 10.3389/fimmu.2022.1033263. 5. Ji R, et al. *Crit Rev Oncol / Hematol.* 2023;186:103993. https://doi.org/10.1016/j.critrevonc.2023.103993. 6. Inamoto Y, et al. *Biol Blood Marrow Transplant*. 2017;23:1250-1256. 7. Wang Z, et al. *Hematol.* 2021;26(1):144-153. 8. Logan BR, et al. *J Clin Invest.* 2023;133(15):e168575. 9. Pratt LM, et al. *Bone Marr Tranpl.* 2013;48(5):722-728.

Potential Biomarkers for cGVHD (cont)

Biomarker	Biologic Function	Prognostic	Diagnostic	Predictive
MMP3	MMP family of proteins regulates the breakdown of extracellular matrix and tissue remodeling[1]; increased MMP3 levels in BOS patients[2]		✓	
MMP9	MMP family of proteins regulates the breakdown of extracellular matrix and tissue remodeling[1]; Increased levels at BOS diagnosis associated with OS[2]	✓		
NK/NK_{reg} cells	Play a key role in tissue fibrosis[3]; along with an imbalance of other immune cell compartments, loss of NK_{reg} cells associates with the future development and severity of cGVHD[4] and different subpopulations may have potential in diagnosing organ-specific cGVHD[3]	✓	✓	
Osteopontin	Osteopontin has broad biological functions, including biomineralization, bone remodeling, and inflammation[5]; detection in plasma as part of a panel with ST2, CXCL9 and MMP3 can predict future cGVHD[6]	✓	✓	
anti-PDGFR	Levels correlate with cGVHD diagnosis and severity; predictive of response to nilotinib for steroid-refractory or steroid-dependent cGVHD[3,7]	✓	✓	✓
PD-1	Inhibitory immune checkpoint receptor; maintains immune tolerance.[3] Levels of soluble PD-1 decreased in cGVHD patients; related to OS, DFS and TRM, and PD-1 expression in circulating CD4+ and CD8+ T cells may be predictive of response to abatacept[3,8]	✓	✓	
Reg3α	Peptide primarily found in Paneth cells of the intestines; increased levels associated with GI cGVHD; increased levels at GI cGVHD diagnosis associated with NRM[2]	✓	✓	
anti-Ro52	Autoantibody against the Ro52 protein; levels found to be higher in patients with active cGVHD than without active cGVHD, and associated with severity of cGVHD; diagnostic potential for skin and liver cGVHD[3,9]	✓		
ST2	Interaction between ST2 and its ligand, IL-33, triggers inflammatory cytokine production and cell proliferation[10]; ST2 levels declined after 2, 4, and 6-months of ECP[2]			✓
Th17 cells	Increased plasma Th17 cells at cGVHD onset; higher levels in allografts predicts high treatment sensitivity[3]		✓	✓
T_{reg} cells	T_{regs} establish tolerance between recipient tissues and donor-derived immunity; decreased T_{reg} in peripheral blood in cGVHD, but enriched in target tissues.[3] Predicts response to ECP in steroid-refractory cGVHD[3]		✓	✓

DFS, disease-free survival; MMP, matrix metalloproteinase; NK_{reg}, regulatory natural killer cell; OS, overall survival; PD-1, programmed cell death protein-1; Reg3α, regenerating islet-derived 3α; ST2, suppression of tumorigenicity 2; TRM, treatment-related mortality.

1. Wang X, Khalil RA. *Adv Pharmacol*. 2018;81:241-330. 2. Bidgoli A, et al. *Transpl Cel Ther.* 2022;28:657-666. 3. Ji R, et al. *Crit Rev Oncol / Hematol.* 2023;186:103993. https://doi.org/10.1016/j.critrevonc.2023.103993. 4. Schultz KR, et al. *Blood*. 2020;135:1287-1298. 5. Icer MA, Gezmen-Karadag M. *Clin Biochem*. 2018;59:17-24. 6. Logan BR, et al. *J Clin Invest.* 2023;133(15):e168575. 7. Chen GL, et al. *Biol Blood Marr Transpl.* 2018;24(2):373-380. 8. Kordelas L, et al. *Bone Marr Transpl.* 2021; 12:3799. 9. Yang K, et al. *Front Immunol.* 2020;11:1505. 10. Reichenbach DK, et al. *Blood*. 2015;125:3183-3192.