Graft-Versus-Host Disease Mechanism of Disease

Presentation Overview

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

SECTION 2 — Acute GVHD

SECTION 3 — Chronic GVHD

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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

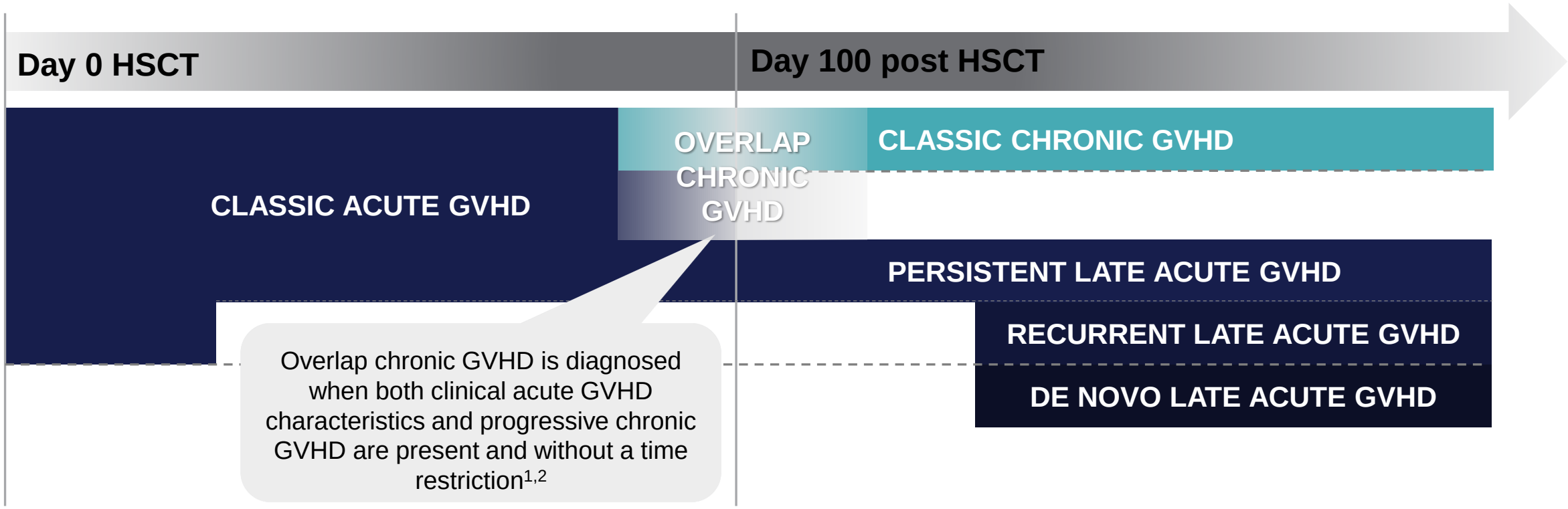
- Complex immune-mediated pathology of cGVHD involves T cells, B cells, macrophages, and fibroblasts[4,5]
- Displays more autoimmune and fibrotic features than aGVHD[4,5]
- Organs affected: primarily oral and ocular mucosal surfaces; may also affect the skin, gut, liver, lungs, and kidneys[2,5]

- 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.

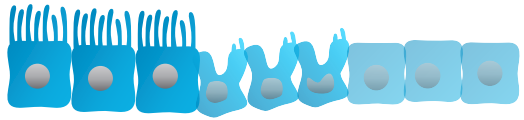
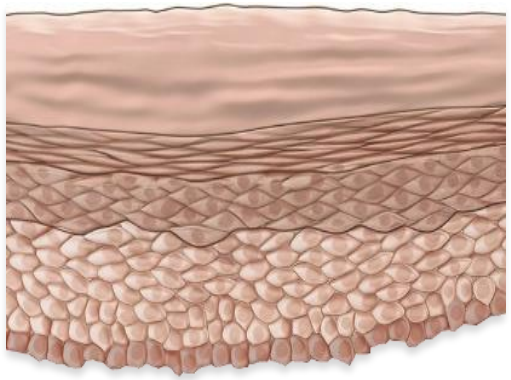
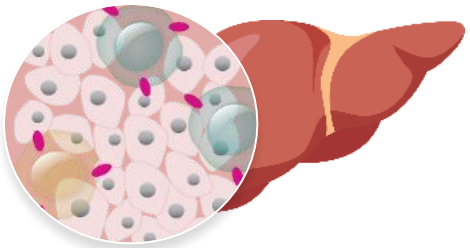
1. Lee SJ. *Blood*. 2017;129:30-37. 2. Filipovich AH, et al. *Biol Blood Marrow Transplant*. 2005;11:945-956.

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]

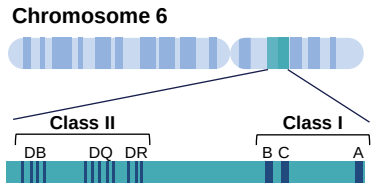
- Steroid-refractory aGVHD has been reported to have a mortality rate of approximately 35%[9]

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.

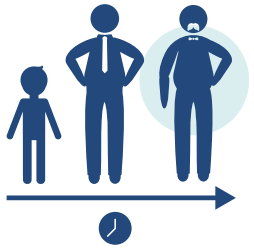
Risk Factors for Development of aGVHD

HLA[1-3]



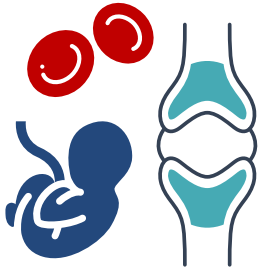
Risk of GVHD is impacted by HLA

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)

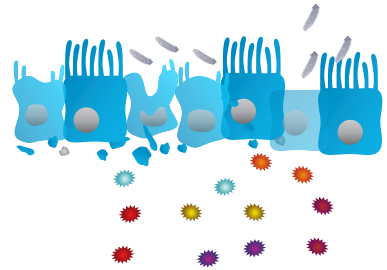
Conditioning regimens[1,7]

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]

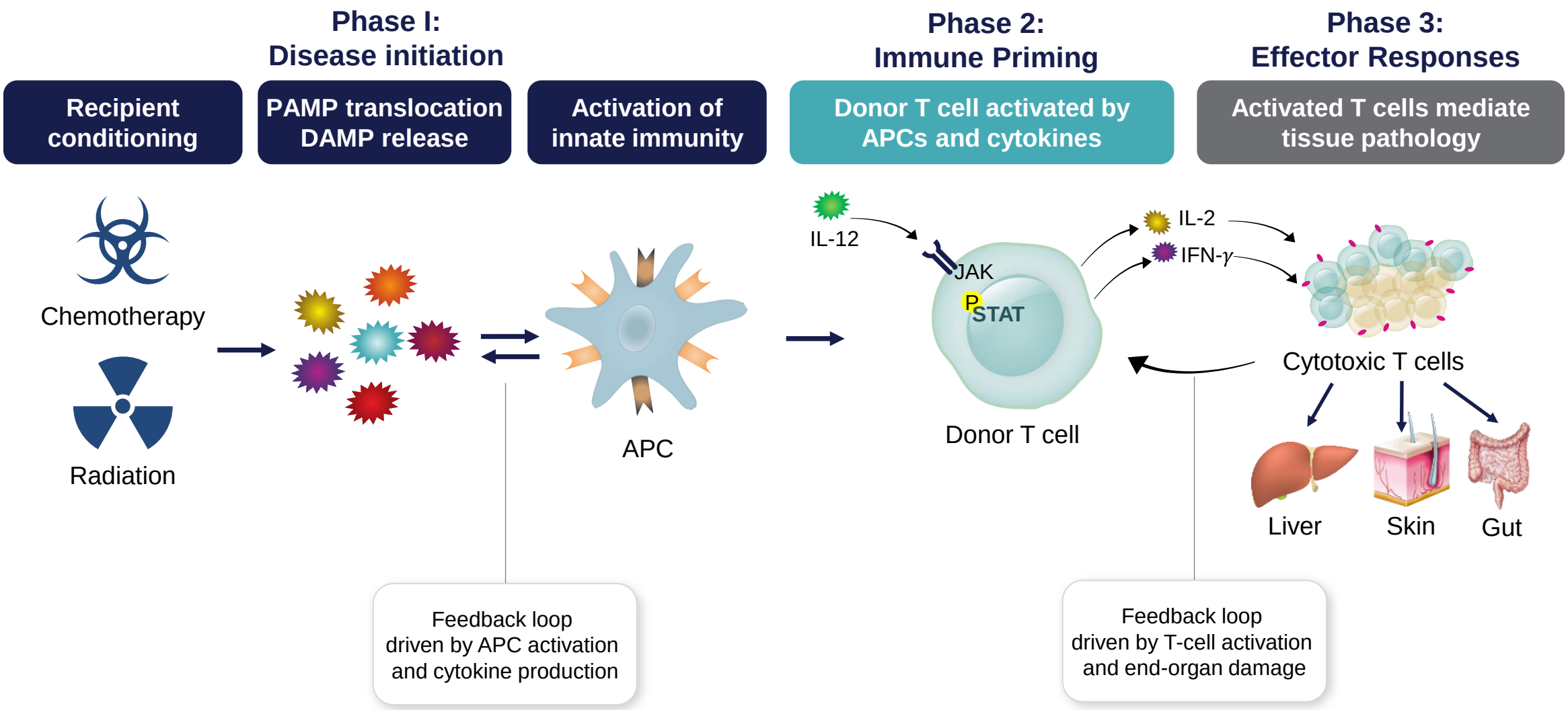


Damage to the GI tract can result in the release of microbial products into systemic circulation, stimulating cytokine production

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



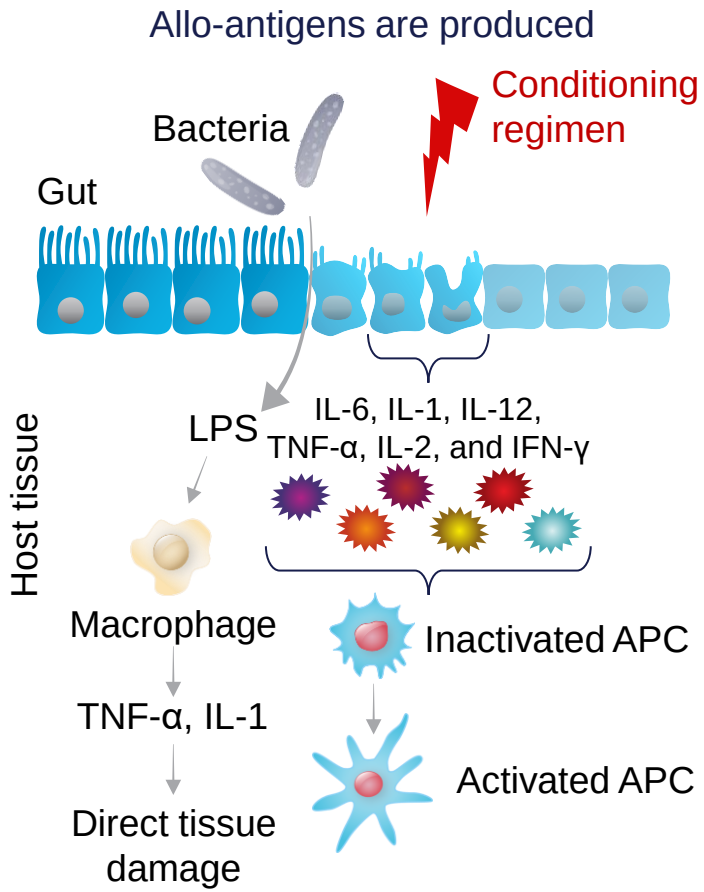
APC, antigen presenting cell; DAMP, damage-associated molecular pattern; IFN, interferon; IL, interleukin; JAK, Janus kinase; P, phosphorylated; PAMP, pathogen-associated molecular pattern; STAT, signal transducer and activator of transcription.

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.

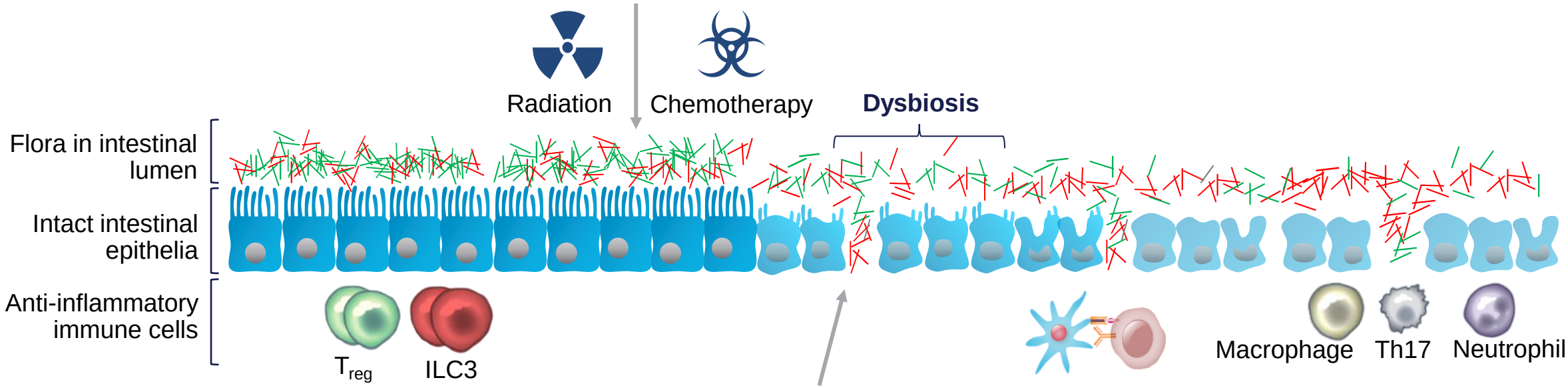
1. Nassereddine S, et al. *Anticancer Res.* 2017;37:1547-1555. 2. McDonald-Hyman C, et al. *Sci Transl Med.* 2015;7:280-282. 3. Ferrara JL, et al. *Stem Cells.* 1996;14:473-489. 4. Blazar BR, et al. *Nat Rev Immunol.* 2012;12:443-458. 5. Toubai T, et al. *Front Immunol.* 2016;7:539. 6. Markey KA, et 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.

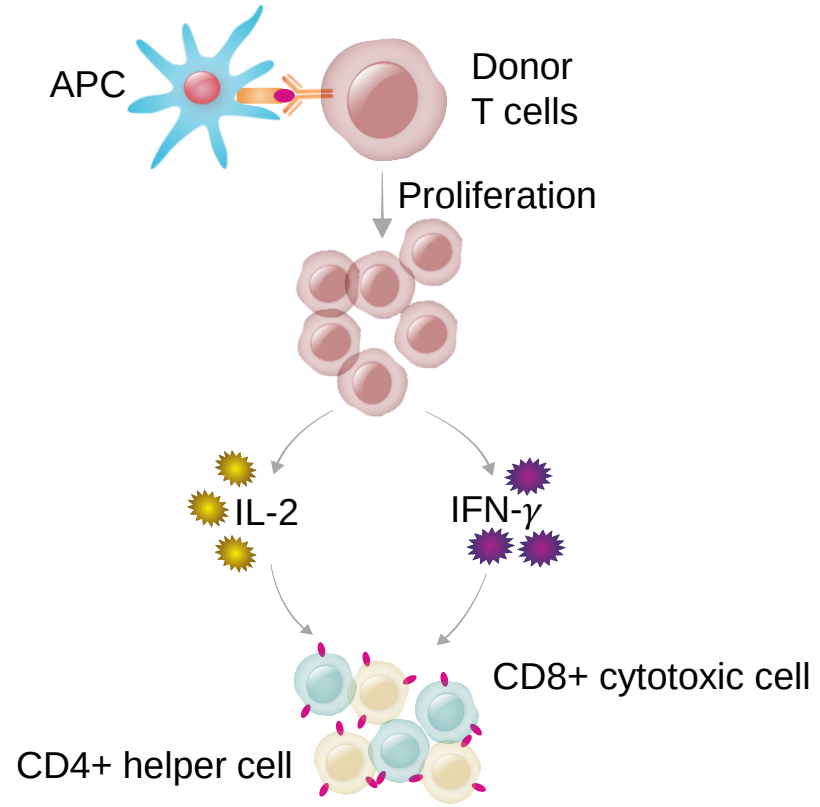
Donor T-Cell Activation and Expansion

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.

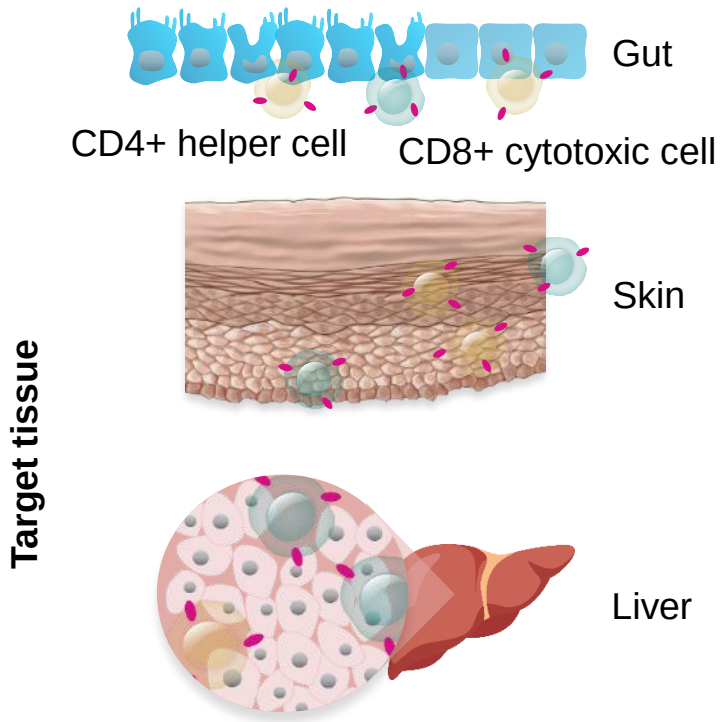
1. Villa NY, et al. *Viruses*. 2016;8:1-30. 2. Abboud R, et al. *Ther Adv Hematol*. 2020;11:1-13. 3. Nassereddine S, et al. *Anticancer Res*. 2017;37:1547-1555. 4. McDonald-Hyman C, et al. *Sci Transl Med*. 2015;7:280-282.

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



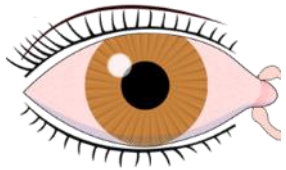
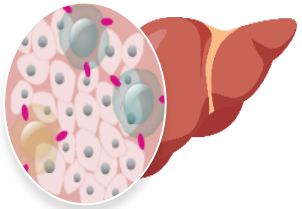
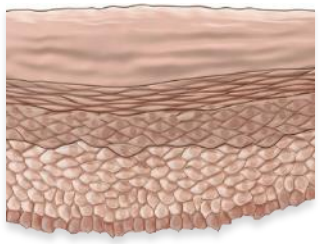
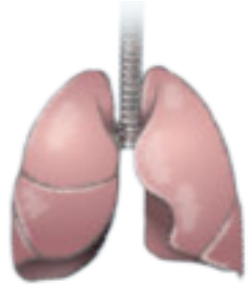
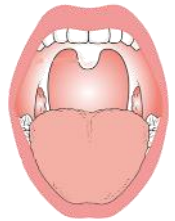
1. Ferrara JLM, et al. *Lancet*. 2009;373:1550-1561. 2. McDonald-Hyman C, et al. *Sci Transl Med*. 2015;7:280-282. 3. Schroeder MA, et al. *Biol Blood Marrow Transplant.* 2018;24:1125-1134. 4. Villa NY, et al. *Viruses*. 2016;8:1-30. 5. Blazar BR, et al. *Nat Rev Immunol.* 2012;12:443-458. 6. Ball LM, 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]

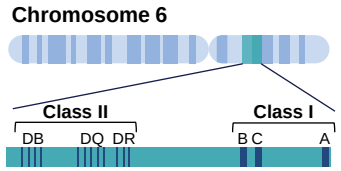
[a] Two-year survival outcomes are based on data from the Fred Hutchinson Cancer Research Center.[3]

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

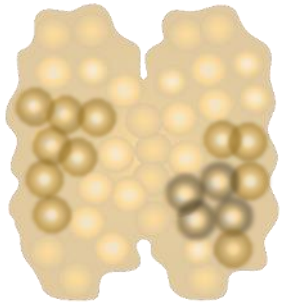
Conditioning regimens[1-3]

Chemotherapy

Radiation

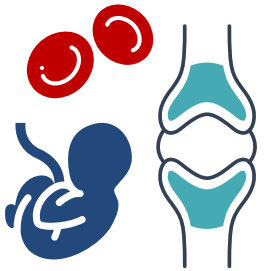
Higher intensity conditioning causes more tissue damage, enhancing cytokine release and immune activation

Prior aGVHD[1,3-5]



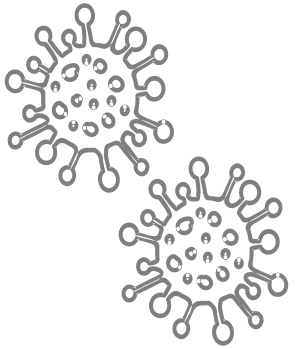
Prior tissue damage from aGVHD, especially to the thymus, may persist and contribute to cGVHD initiation

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

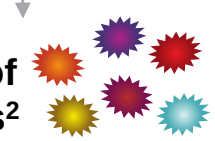
Disease initiation[1]

Chemotherapy

Radiation

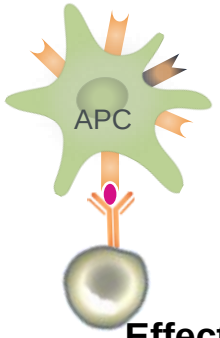
Host Tissue Damage

Release of Cytokines[2]

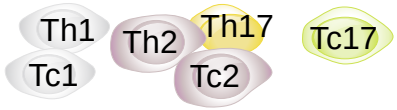


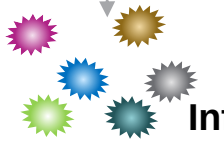
T-cell phase[3]

T-cell Activation



Effector T-Cell Differentiation

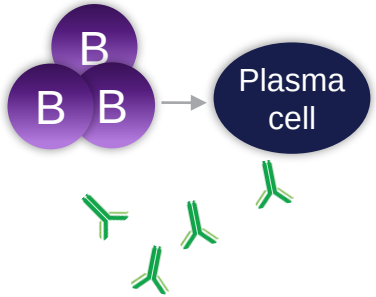




Chronic Inflammation

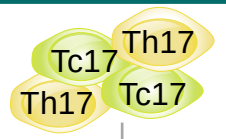
B-cell Phase[2,3]

Aberrant B-cell activation/expansion



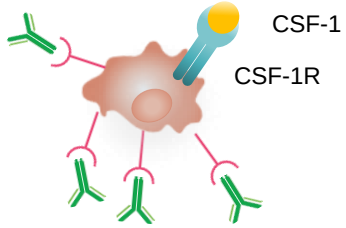
Constitutive allo/autoantibody production

Macrophage/fibrosis phase[3]

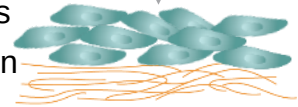


CSF-1, IFN-γ, IL-22, TNF, IL-13, IL-17

Macrophage Survival and Activation



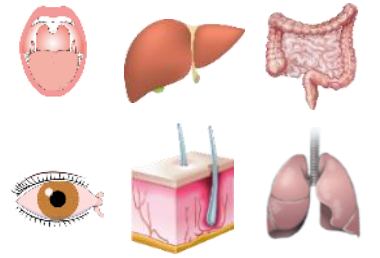
TGF-β



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

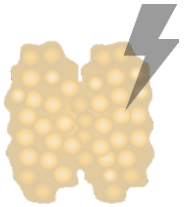
Chemotherapy

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]

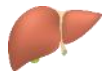


Thymus

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

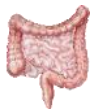
Mouth

Eyes

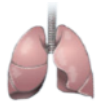


Liver

Skin



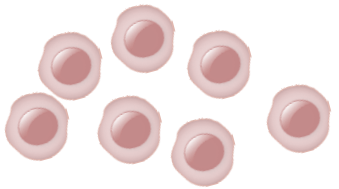
Gut



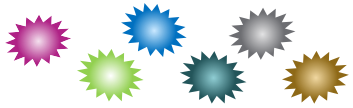
Lungs

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.

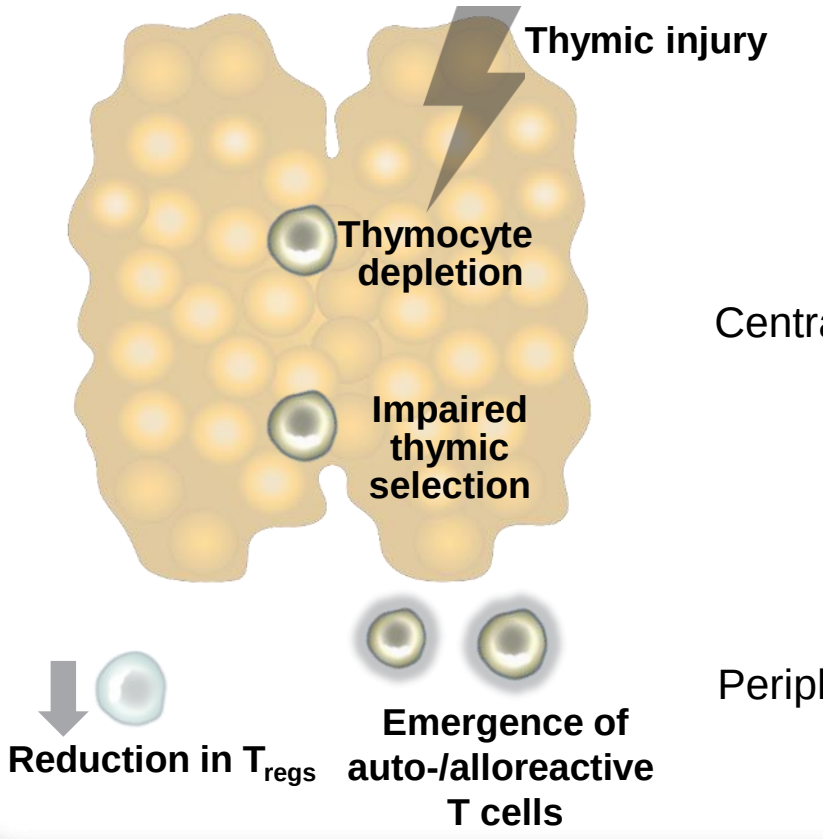
Dysregulated T-Cell Immunity: Thymic Dysfunction Contributes to cGVHD

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]

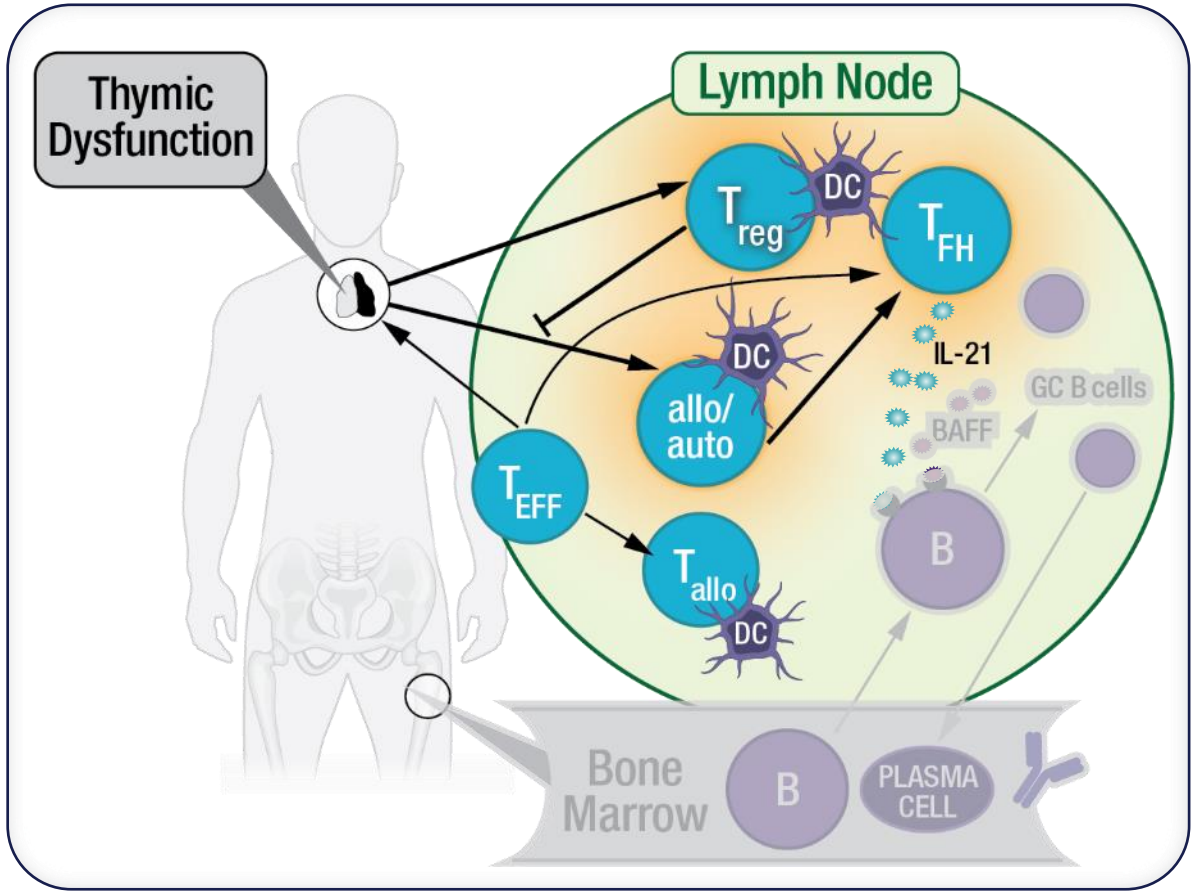


1. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 2. MacDonald KPA, et al. *Blood*. 2017;129:13-21. 3. Wu T, et al. *J Immunol*. 2013;191:488-499. 4. Zeiser R, Blazar BR. *N Engl J Med*. 2017;377:2565-2579. 5. McDonald-Hymen C, et al. *Sci Transl Med*. 2015;7:280-282. 6. Beres AJ, 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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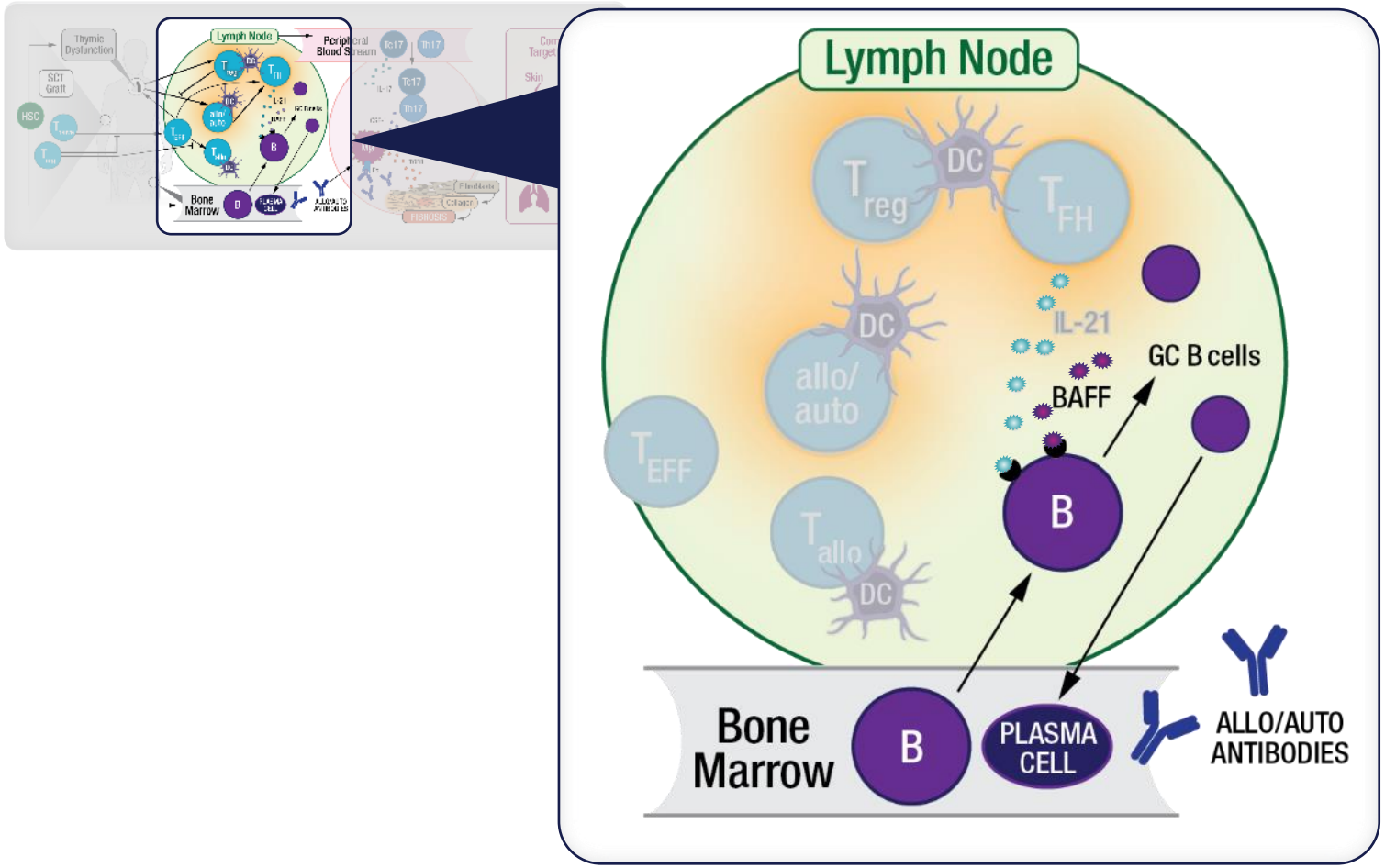
DC, dendritic cell; T_{EFF}. effector T cell; T_{FH}, T follicular helper cell.

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



- 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.

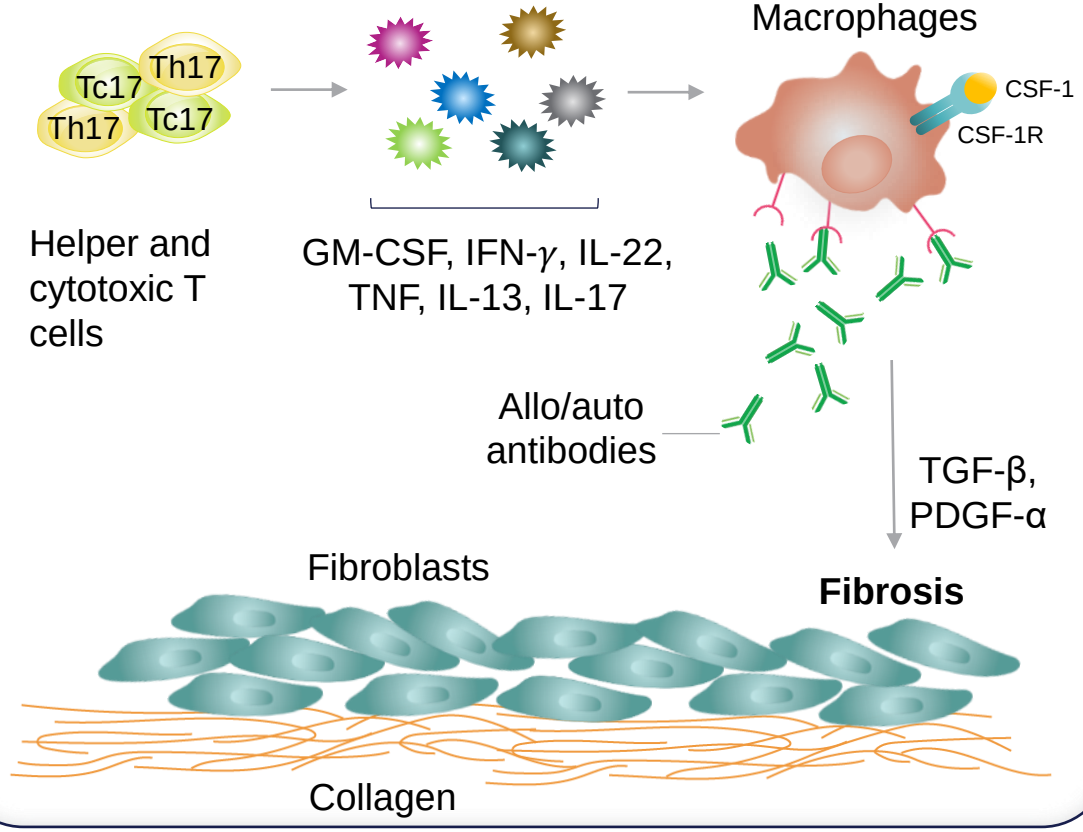
1. Sarantopoulos S, et al. *Biol Blood Marrow Transplant*. 2015;21:16-23. 2. Shimabukuro-Vornhagen A, et al. *Blood*. 2009;114:4919-4927. 3. Cooke KR, et al. *Biol Blood Marrow Transplant*. 2017;23:211-234. 4. Schroeder MA, DiPersio JF. *Dis Model Mech*. 2011;4:318-333. 5. MacDonald KPA, et al. *Blood*. 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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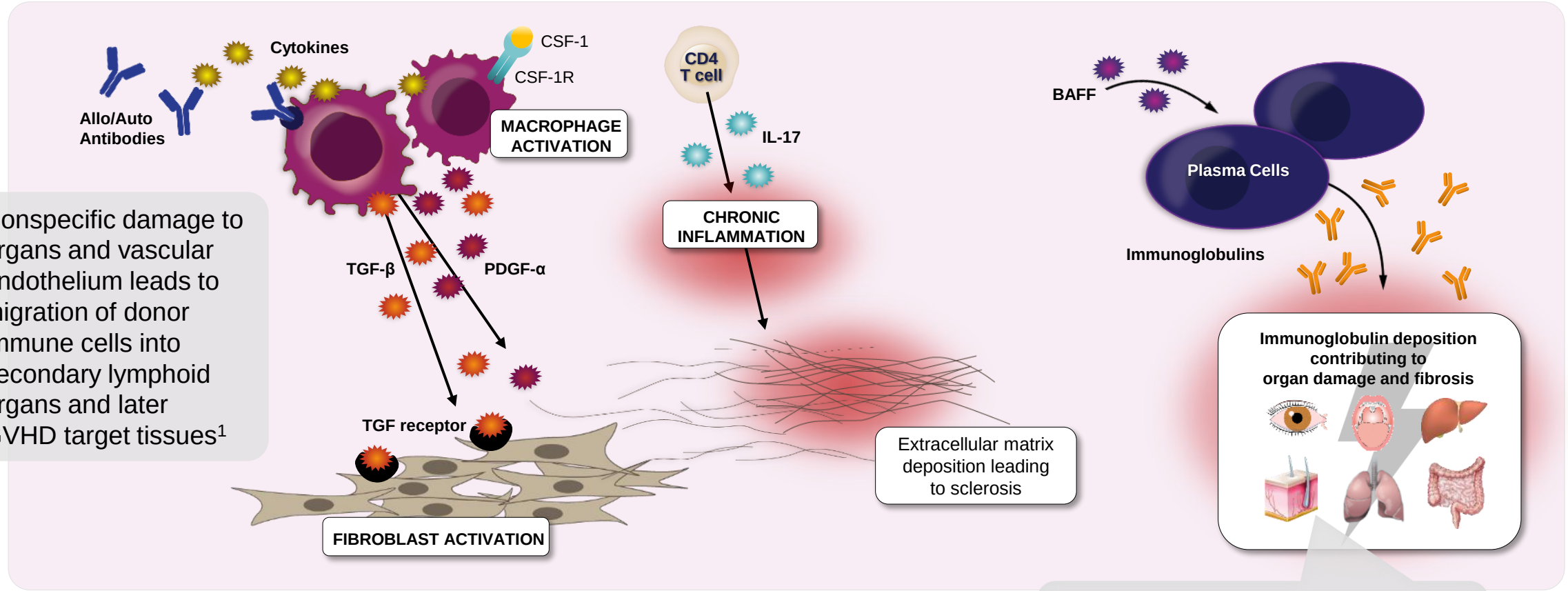
GM-CSF, granulocyte-macrophage colony-stimulating factor; PDGF-α, platelet-derived growth factor alpha.

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]



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.

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