

Acute GVHD: *Diagnosis and Management*

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Learning Objectives

Pathophysiology

Understand the three-phase model of acute GVHD development

Diagnosis

Master clinical examination and biopsy interpretation

Management beyond 1st line

Manage steroid-refractory disease with ruxolitinib

Clinical Recognition

Recognize presentations and apply MAGIC staging criteria

Therapeutic Strategy

Apply evidence-based first-line and salvage treatments

Treating beyond what meets the eye

Identify and manage treatment-related complications

Definition and Clinical Classification

What is Acute GVHD?

Acute GVHD results from recognition and destruction of recipient tissues by immunocompetent donor T cells. It is a major immune complication after allogeneic hematopoietic cell transplantation.



Key Insight: aGVHD is the leading contributor to non-relapse mortality post-transplant, affecting up to 62% of patients despite prophylaxis (BMT CTN 1202).

NIH Consensus Criteria (2005)

- **Classic acute GVHD:** Onset within 100 days post-transplant
- **Late-onset aGVHD:** First episode >100 days
- **Recurrent aGVHD:** New episode >100 days with prior classic aGVHD history
- **Persistent aGVHD:** Symptoms persist >100 days

Filipovich, A. H. 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. 11, 945–956 (2005).

Target Organs and Epidemiology

Skin

Most common presentation (50-60% of cases).
Maculopapular rash typically at engraftment
and beyond (first 100d classically).



GI Tract

30-50% involvement.
Diarrhea, abdominal pain, and protein-losing
enteropathy.

Liver

5-10% isolated involvement.
Cholestatic pattern with hyperbilirubinemia.

Critical Statistics

- Grade II-IV aGVHD incidence: **49-62%** despite prophylaxis
- Grade III-IV aGVHD incidence: **19-40%**
- Median onset: **14-21 days** post-transplant (at engraftment)
- 1-year OS with Grade III-IV: **40%** (vs 70% for Grade II)

□ **Good News:** Grade III-IV aGVHD has decreased from 40% (1990-1995) to 28% (2011-2015) with improved conditioning and prophylaxis.

The Three-Phase Model of aGVHD

Phase 1: Initiation



Conditioning-induced tissue damage releases PAMPs and DAMPs, activating host APCs and creating inflammatory milieu.

Phase 2: T Cell Activation



Alloreactive donor T cells recognize host MHC antigens > differentiate to Th1 phenotype > expand.

Phase 3: Effector Phase



CTLs kill target cells via multiple pathways while cytokine storm amplifies tissue destruction.

Phase 1: The Inflammatory Foundation

Conditioning-Induced Damage

High-dose chemotherapy and total body irradiation cause direct epithelial injury to intestinal mucosa, skin, and liver. Damage intensity correlates with aGVHD severity through increased TNF- α production.


Danger Signal Release

- PAMPs: LPS, bacterial DNA stimulate TLR4, NOD2, RIG-I pathways
- DAMPs: HMGB1, uric acid, ATP, IL-33, heat shock proteins

Soluble ST2 (IL-33 receptor) serves as a biomarker for aGVHD severity and therapy resistance.

Microbiota's Critical Role

Conditioning and antibiotics reduce gut microbiota diversity. Loss of protective bacteria (Blautia, Clostridiales) producing short-chain fatty acids leads to barrier dysfunction.

 **Clinical Implication:** Enterococcus dominance correlates with poor outcomes. Microbiota preservation reduces aGVHD lethality.

Phase 2: T Cell Recognition and Differentiation

Alloreactive Recognition

- **CD4+ T cells:** Recognize host MHC class II (HLA-DR, DQ, DP) on APCs
- **CD8+ T cells:** Recognize host MHC class I (HLA-A, B, C)
- **Major antigens:** HLA mismatch is primary GVHD driver
- **Minor antigens:** HA-1, HA-2, HA-4 important in HLA-matched transplants

Th1 Polarization

CD4+ T cells differentiate toward Th1 phenotype, producing IL-2 and IFN- γ , expanding CTLs and NK cells.

Regulatory Mechanisms

Natural and induced Tregs provide suppression, but thymic damage from conditioning impairs their generation. Regulatory B cells, MDSCs, and ILC2/ILC3 also contribute to immune regulation.

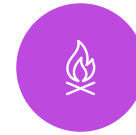
mTOR inhibitors can stimulate CD8+ Treg cells, offering therapeutic potential.

Phase 3: Tissue Destruction and Cytokine Storm



Cellular Cytotoxicity

CTLs kill via Fas-FasL, perforin-granzyme, TRAIL pathways. Necroptosis via RIPK1 causes inflammation-associated cell death.



Cytokine Storm

IFN- γ , TNF- α , IL-2, IL-6 production by activated Th1 cells and macrophages creates systemic inflammation.



Organ-Specific Damage

Skin: Keratinocyte apoptosis. **GI:** Crypt epithelial and Paneth cell loss. **Liver:** Bile duct epithelial apoptosis.



Barrier Dysfunction

Tight junction disruption increases permeability, causing bacterial translocation and cytokine amplification loop.

Major Risk Factors for Acute GVHD

Evidence from CIBMTR Analysis (Largest Multicenter Trial)

Unrelated donor (vs related)	1.61 (1.54-1.67)	<0.001
Disease not in CR	1.25 (1.2-1.3)	<0.001
Bone marrow source	1.2 (1.15-1.25)	<0.001
Female donor → Male recipient	1.16 (1.11-1.21)	<0.001
ATG/Alemtuzumab (protective)	0.79 (0.74-0.84)	<0.001

Conditioning Intensity

- MAC: Highest risk
- RIC: Lower risk
- Non-myeloablative: Lowest but still occurs

HLA Disparity Impact

- MRD: Baseline risk
- MUD: HR 1.6-2.0
- MMUD: Highest risk
- PTCy: Overcomes mismatch

Other Factors

- Donor age >30 years
- Recipient older age
- Active disease at transplant

Clinical Diagnosis: Skin Acute GVHD

Clinical Presentation

First manifestation in ~70% of patients, typically at engraftment (14-21 days).


Presents as pruritic, painless, maculopapular rash starting in sun-exposed areas—nape, shoulders, palms, soles—spreading progressively.

Staging Progression

1. **Stage 1:** Scattered maculopapules, <25% BSA
2. **Stage 2:** Generalized erythema, 25-50% BSA
3. **Stage 3:** Marked erythema, >50% BSA
4. **Stage 4:** Bullae, desquamation, mucosal involvement

Histopathology Hallmarks

- Basal layer degeneration with keratinocyte loss
- Apoptotic bodies (dyskeratosis) with satellite lymphocytes
- Perivascular lymphocytic infiltration in dermis
- Dermoepidermal disjunction in severe cases
- Mild-moderate inflammation without extensive infiltration

 **Biopsy Tip:** Histology supports clinical diagnosis but should NOT delay treatment initiation.

Clinical Diagnosis: GI Acute GVHD

Lower GI Presentation

Hallmark: Secretory, voluminous diarrhea (often >500 mL/day in severe disease). Accompanied by abdominal pain, ileus in severe cases, and hematochezia indicating mucosal ulceration.

Protein-Losing Enteropathy

Plasma protein loss through damaged mucosa manifests as decreased albumin *before* symptoms. Elevated fecal α -1 antitrypsin predicts progression to severe aGVHD.

Upper GI Presentation

- Persistent anorexia (>3 days)
- Nausea and frequent vomiting (≥ 2 episodes/day)
- Often occurs without lower GI symptoms
- Better prognosis than lower GI or combined disease

Critical Differential Diagnosis

Must exclude: C. difficile (most important), CMV, viral infections, conditioning toxicity, MMF toxicity, neutropenic enterocolitis

Diagnostic Approach

1. Infectious workup first (stool studies, CMV PCR)
2. Endoscopy with biopsies after negative infectious studies
3. Upper endoscopy: 4-6 biopsies from erosions + pyloric area
4. Sigmoidoscopy: Rectosigmoid biopsies even if normal-appearing

GI GVHD: Endoscopy and Histology

Endoscopic Findings

- Spotted or diffuse erythema
- Patchy erosions and scattered ulcerations
- Active bleeding
- **May appear completely normal despite significant GVHD**

Histologic Hallmarks


- **Apoptotic epithelial cells at crypt base (hallmark)**
- Individual or multiple crypt loss
- Epithelial denudation
- Neutrophilic infiltration (severe disease)
- Increased eosinophils (worse prognosis)

Diagnostic Challenge

GVHD is patchy—negative biopsy doesn't exclude disease.

Clinical + endoscopic + histologic correlation is diagnostic gold standard.

Often must treat based on high clinical suspicion + abnormal findings.

 **Key Point:** Low Paneth cell numbers correlate with increased mortality. Enhanced endoscopy techniques (CT enterography, FDG-PET) are emerging diagnostic tools.

Hepatic GVHD: Clinical Presentation

Hyperbilirubinemia is the primary finding, with jaundice in severe cases. Elevated alkaline phosphatase and GGT indicate predominant duct injury. **Least commonly involved organ in aGVHD, usually associated with skin and/or GI involvement.**



Diagnosis Often by Exclusion: Must rule out SOS, viral hepatitis, drug-induced liver injury, cholangitis lenta.

Hepatic GVHD: Three Injury Patterns

01

Cytokine-Induced Cholestasis (Early)

IL-6 and TNF- α affect hepatocyte bilirubin transporters. Normal bile ducts on biopsy. Reversible with treatment.

02

Bile Duct Injury

Lymphocytic infiltration of small ducts with epithelial dropout. Zone 3 cholestasis. Severe: ductopenia. Usually accompanies GI GVHD.

03

Acute Hepatitis

Marked transaminase elevation. Usually after day 100 (not classic aGVHD). Seen with minimal immunosuppression or post-DLI.

MAGIC Grading System

Standardized Staging and Grading Criteria

0	No rash	Normal	None	<1.5 mg/dL
1	≤25% BSA	500-1000 mL/d	Mild anorexia/nausea	1.5-2.5 mg/dL
2	25-50% BSA	1000-1500 mL/d	Mod. symptoms, vomit 1x/d	2.5-6 mg/dL
3	>50% BSA	>1500 mL/d	Severe, vomit 2-5x/d	6-15 mg/dL
4	Bullae/desquamation	>1500 mL/d + bleeding	Req. IV alimentation	>15 mg/dL

Grade I (Mild)

Stage 1 any organ only

Grade II (Moderate)


Stage 2 any organ

Grade III (Severe)

Stage 3 skin/GI, OR stage 2 liver + stage 1 elsewhere, OR stage 3 liver

Grade IV (Very Severe)

Stage 4 any organ

 **Grading Rule:** Overall MAGIC grade based on HIGHEST organ stage involved.

Prognostic Biomarkers

Research-Grade Composite Panel

IL-2R, TNF receptor 1, IL-8, HGF panel achieves AU-ROC 0.91 (95% CI 0.87-0.94) for distinguishing aGVHD from no aGVHD at GI onset.

Ann Arbor Score

Multibiomarker panel (IL-2R, sTNFR1, REG3 α , IL-8) predicts response to initial therapy and overall survival. Identifies patients requiring alternative therapy by day 28.

Individual Biomarkers

- **ST2 (soluble):** High levels \rightarrow therapy-resistant aGVHD, increased mortality
- **REG3 α :** GI-specific injury marker, elevated in gut GVHD
- **TIM-3 (soluble):** Associated with severe aGVHD
- **sTNFR1:** Systemic inflammation marker
- **Fecal α -1 antitrypsin:** Predicts grade 3-4 disease
- **Fecal calprotectin:** Reflects mucosal inflammation

Biomarkers are prognostic at GVHD onset and guide treatment intensity, but should not delay therapy initiation.

First-Line Treatment: Strategic Approach

Grade I Skin-Only

Topical steroids alone (fluticasone 0.05%, triamcinolone 0.1%) 2-3x daily. Most resolve by day 20-30.

AVOID STEROIDS at initiation

1

2

Grade II (Skin ± Upper GI, No Lower GI/Liver)

Low-dose corticosteroids: Methylprednisolone 0.5-1 mg/kg/day. Equivalent efficacy to 2 mg/kg with no compromise in survival. Minimum 7 days at starting dose before taper.

3

Grade II-IV with GI or Hepatic Involvement

Standard-dose corticosteroids: Methylprednisolone 2 mg/kg/day IV or oral. IV preferred if vomiting/malabsorption. Add non-absorbable steroids for GI: Budesonide 9 mg/day.

4

Key Principle

NO additional immunosuppressive agents with first-line steroids. Meta-analysis: Adding MMF, ATG, infliximab, or anti-IL2 → 14% survival DECREASE. Save second-line for steroid-refractory disease.

Ruxolitinib: The JAK2 Inhibitor Revolution

Mechanism of Action

- Selective JAK2 inhibitor blocking STAT1 and STAT3 phosphorylation.
- - Suppresses T cell activation, Th1 differentiation, pro-inflammatory cytokines (IFN- γ , TNF- α , IL-6),
- Preserving Treg function and anti-tumor immunity.

REACH 2 Trial: Landmark Evidence

Phase III randomized trial in steroid-refractory acute GVHD comparing ruxolitinib vs best available therapy.

62%

Overall Response Rate (Day 28)

vs 39% with BAT (p<0.001)

40%

Durable Response (Day 56)

Superior to BAT

2020

FDA/EMA Approval Year

Category 1 NCCN & EBMT

❏ **Definition Steroid-Refractory:** Progressive/stable disease despite 2 mg/kg/day steroids for ≥ 3 days. Declare early (day 3-5) if rapidly progressing. Affects 50-75% of Grade III-IV patients.

Ruxolitinib: Dosing and Monitoring



Standard Dosing

10 mg orally twice daily. Adjust based on clinical response and tolerability. Consider IV formulation if severe diarrhea (>1500 mL/day) reduces absorption.



Special Considerations

Use caution with platelets <30,000/ μ L or ANC <500/ μ L. Monitor for drug interactions with CYP3A4 inhibitors/inducers (adjust dose with azole antifungals).



Response Timeline

Day 3-7: Early assessment.

Day 14-28: Primary efficacy endpoint. Continue if responding or disease stabilized. CR = resolution of all signs; PR = improvement without worsening.



Toxicity Monitoring

CBC at baseline, then **weekly \times 4 weeks**, then as indicated. LFTs periodic monitoring. Lipid profile at baseline and during therapy. Similar infection rate to BAT.

Key Advantage: Large beneficial effect on response and failure-free survival with no excess toxicity compared to best available therapy.

Beyond Ruxolitinib: Third-Line Options

❏ **Critical Recommendation:** No FDA-approved standard third-line therapy. **Strongly recommend clinical trial enrollment** for ruxolitinib-refractory disease. Non-relapse mortality remains 50-70%.

Cellular Therapies

Extracorporeal Photopheresis (ECP): 40-60% response in skin/GI GVHD. Well-tolerated, synergistic with other therapies.

MSC Therapy: Ongoing Phase III IDUNN trial (MC0518) for pediatric SR-aGVHD.

Emerging Agents

Alpha-1 Antitrypsin (CSL964): Phase III ongoing. **MaaT013 Microbiota:** 62% GI response by Day 28 in Phase III ARES trial (Dec 2024). **Vedolizumab:** Selective $\alpha 4\beta 7$ integrin for GI-predominant disease.

Salvage Options

Anti-TNF (Infliximab): 40-50% response in GI aGVHD, increased fungal risk. **ATG/Alemtuzumab:** ~30% response, profound immunosuppression. **MMF, Pentostatin:** 30-40% response in combinations.

Supportive Care: The Foundation of Success

Infection Prophylaxis Essentials

Fungal Prevention

Posaconazole 300 mg/day (superior to fluconazole for Aspergillus/Candida).

Strategy 1 : Stop at engraftment

Strategy 2 : Continue throughout high immunosuppression until CD4 >50-100 cells/mm³.

CMV Surveillance

Quantitative PCR weekly/biweekly. Preemptive ganciclovir 5 mg/kg IV BID if positive without symptoms.

Foscarnet for GCV-resistant.

Letermovir prophylaxis for *high-risk* patients.

PCP Prophylaxis

TMP-SMX double-strength daily (also covers Toxoplasma).

Alternatives: *pentamidine IV monthly*, dapsone + pyrimethamine.

Continue ≥1 month after steroid taper.

EBV/PTLD Prevention

EBV PCR weekly/biweekly. If rapidly rising (>1 log/week) → rituximab 375 mg/m² weekly × 4 significantly reduces PTLD development.

Steroid Toxicity Management

Hyperglycemia

Daily glucose monitoring, insulin if >180 mg/dL

Osteoporosis

Calcium 1200 mg/day, Vitamin D 800-2000 IU, bisphosphonates if steroids >3-4 weeks

Avascular necrosis

Minimize steroid exposure, baseline MRI hips/knees if risk factors

Muscle wasting

Early mobilization, physiotherapy, adequate protein

GI protection

PPI or H2-blocker for stress ulcer prophylaxis

Prolonged corticosteroid exposure is an independent risk factor for mortality—taper as rapidly as safely possible.

Nutritional and GI Support



Total Parenteral Nutrition (TPN)

Essential for severe GI GVHD with malabsorption. Maintain until oral intake >50% of needs and diarrhea controlled



Bowel Rest

Complete bowel rest during acute severe GI GVHD reduces antigen exposure and allows mucosal healing



Gradual Refeeding

Start with elemental/semi-elemental formulas. Progress slowly to low-residue diet as tolerated



Fluid and Electrolyte Management

Aggressive replacement for high-volume diarrhea. Monitor magnesium, potassium, and phosphate closely



Pain Management

Adequate analgesia for abdominal cramping—avoid NSAIDs due to bleeding risk

Case Scenario

A 42-year-old woman undergoes allogeneic hematopoietic stem cell transplantation (HSCT) from a matched unrelated donor (MUD) for acute lymphoblastic leukemia (ALL).

She receives myeloablative conditioning (busulfan/cyclophosphamide) and peripheral blood stem cell transplant.

GVHD prophylaxis consists of tacrolimus and short-course methotrexate.

Day 28 post-transplant presentation:

- Well-engrafted (ANC $>1000/\mu\text{L}$, platelets $>50,000/\text{L}$)
- Chief complaint: Persistent anorexia, nausea, and bilious vomiting (2-3 episodes per day)
- Physical examination: Faint maculopapular rash on trunk (~15% BSA); normal abdominal examination
- No diarrhea; stool output normal
- Laboratory studies:
 - Total bilirubin: 1.2 mg/dL (normal)
 - ALT: 45 U/L (normal)
 - Serum albumin: 3.2 g/dL (mildly decreased from 3.8 g/dL)
 - CMV PCR: Negative
 - Stool studies: C. difficile toxin negative, viral culture negative

Diagnostic workup considerations: The patient's symptoms are concerning for upper gastrointestinal GVHD, but her clinician must rule out other etiologies. Upper endoscopy with biopsy is being considered.

Question 1

Which of the following is the MOST IMPORTANT principle regarding the diagnostic approach to suspected upper GI acute GVHD in this patient?

- A. Delay initiating treatment until biopsy results confirm upper GI GVHD histologically, as false-positive diagnosis is common and unnecessary steroids increase infection risk.
- B. Begin treatment immediately based on clinical suspicion (persistent symptoms after day 20, compatible presentation, negative infection workup), and use biopsies to support rather than confirm the diagnosis.
- C. Perform endoscopy without biopsy; if edematous mucosa is visible, this is sufficient to diagnose GVHD; biopsies are unnecessary if endoscopy is typical.

Case Scenario

A 28-year-old male undergoes allogeneic HSCT from an HLA-mismatched unrelated donor (MMUD, one antigen mismatch) for AML in second remission. He receives myeloablative conditioning with total body irradiation (TBI) 12 Gy and cyclophosphamide.

Day 27 post-transplant:

- Develops acute GVHD with severe lower GI involvement:
 - Diarrheal volume: 1800 mL/day (secretory)
 - Severe colicky abdominal pain
 - Diffuse rash on trunk and extremities (~60% BSA, Stage 3)
 - Total serum bilirubin: 5.8 mg/dL
 - Serum albumin: 2.0 g/dL (decreased from 3.5 g/dL pre-transplant)

Diagnosis: Grade III acute GVHD (Stage 3 skin, Stage 3 lower GI, Stage 2 liver)

Initial treatment (Day 28):

- Methylprednisolone 2 mg/kg/day IV (starts day 28)
- Beclomethasone 8 mg/day (topical steroid for GI)
- Supportive care: IV fluids, TPN initiated

Case Scenario (continued)

Day 35 assessment (Day 7 of steroid therapy):

- Diarrheal volume: 1600 mL/day (minimal improvement)
- Abdominal pain: Unimproved
- Rash: Unchanged (still ~60% BSA)
- Bilirubin: 5.2 mg/dL (slight improvement, but still significantly elevated)
- Patient is becoming increasingly bed-bound and cachectic
- Blood cultures negative; CMV PCR negative
- No active infection identified

Clinical judgment: The patient shows signs of steroid-refractory acute GVHD by Day 7.

Question 2

What is the MOST APPROPRIATE next therapeutic intervention for this steroid-refractory Grade III acute GVHD?

- A. Escalate methylprednisolone dose to 5 mg/kg/day, as the current dose may be subtherapeutic. Add mycophenolate mofetil (MMF) 1.5 g three times daily to the regimen for synergistic immunosuppression.
- B. Continue current steroid regimen and add antithymocyte globulin (ATG) at 4-5 mg/kg total over 3-5 days.
This combination of intensified T cell depletion is the standard of care for steroid-refractory aGVHD.
- C. Initiate ruxolitinib 5 mg orally twice daily WHILE continuing methylprednisolone 2 mg/kg/day. Monitor for response at Day 14-28.
Plan gradual steroid taper if responding.
- D. Switch to high-dose tacrolimus (target level 15-20 ng/mL) and discontinue corticosteroids entirely, as prolonged steroid exposure is contributing to his cachexia and infection risk.
- E. Refer for extracorporeal photopheresis (ECP) as monotherapy (discontinue steroids), which induces immune tolerance without the infection risks of systemic immunosuppression.

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Fellowship in Clinical Hematology and BMT

- *1 year training in BMT and Cellular therapies*
- *Exposure to all aspects of clinical hematology and BMT*
- *Focused advanced training in stem cell transplant and cellular therapies for post-DM/DrNB physicians*
- *Exposure to clinical research methodology and introduction to clinical trials*
- *Eligibility:*
 - *Advanced fellowship: DM / DrNB (Clinical Hematology / Medical Oncology)*



Thank You!

Happy to take questions!