Journal of Research Administration
Society of Research Administrators International

DRUG DOSAGE REGIMEN FOR GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS IN STEM CELL TRANSPLANTATION: A REVIEW

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Abstract
Graft-versus-host disease (GVHD) is a life-threatening immunological complication that frequently follows allogeneic hematopoietic stem cell transplantation (allo-HSCT), a curative option for several hematological malignancies and non-malignant disorders. GVHD arises when donor-derived T lymphocytes recognize the recipient's tissues as foreign, leading to systemic inflammation and organ dysfunction. It is clinically categorized into acute and chronic forms, each with distinct etiopathological features. Preventive strategies aim to mitigate GVHD while preserving graft-versus-leukemia (GVL) effects. This review summarizes the pathogenesis, classification, risk factors, and evolving pharmacological approaches used for GVHD prophylaxis, emphasizing their mechanisms and clinical outcomes.

Keywords: Circular Economy, E-waste Management, Extended Producer Responsibility, India, National Green Tribunal

1. INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains a cornerstone treatment for high-risk hematologic diseases. However, GVHD significantly limits its long-term success, contributing substantially to non-relapse morbidity and mortality [1]. GVHD results from immunological incompatibility between donor and recipient, particularly due to disparities in human leukocyte antigens (HLA), wherein donor T cells attack host tissues [2].

Acute GVHD (aGVHD) typically presents within 100 days of transplantation, targeting the skin, gastrointestinal tract, and liver. Symptoms include erythematous rash, profuse diarrhea, and cholestasis [3]. Chronic GVHD (cGVHD), developing later, can arise de novo or from preceding aGVHD and mimics autoimmune diseases, affecting mucocutaneous, ocular, pulmonary, and hepatic systems [4].

Despite advances in HLA typing, graft manipulation, and immunosuppressive regimens, GVHD remains a principal challenge in allo-HSCT, emphasizing the need for personalized and effective prophylaxis.

2. PATHOPHYSIOLOGY OF ACUTE AND CHRONIC GVHD

a). Acute GVHD (aGVHD)

The pathogenesis of aGVHD involves a triphasic process initiated by conditioning-related tissue injury. This triggers the release of pathogen-associated (PAMPs) and damage-associated molecular patterns (DAMPs), which activate antigen-presenting cells (APCs) and stimulate the release of pro-inflammatory cytokines such as IL-6, IL-12, and IFN-γ [5]. This pro-inflammatory environment primes donor T cells, which migrate to target organs and elicit cytotoxic responses, particularly via Th1 and Th17 pathways [6].

A pivotal event is the upregulation of major histocompatibility complex (MHC) antigens on host cells, facilitating allo-recognition by donor T cells. The final effector phase is characterized by T cell-mediated cytotoxicity, leading to epithelial cell apoptosis and tissue destruction in the skin, gut, and liver [7].

b). Chronic GVHD (cGVHD)

Unlike the acute variant, cGVHD is driven by dysregulated adaptive immunity, particularly involving impaired central and peripheral tolerance. Thymic injury, T regulatory (Treg) cell depletion, aberrant B-cell activation, and fibrotic changes define its pathogenesis [8,9]. Three overlapping stages are recognized: (i) initial tissue damage and persistent inflammation, (ii) immune dysregulation with pathogenic Th17/Tc17 and T follicular helper (Tfh) cell expansion, and (iii) progressive fibrosis, especially in the skin and lungs [10]. Fibrosis is mediated by cytokines such as TGF-β and PDGF, which stimulate fibroblast activation and collagen deposition. Macrophage polarization into pro-fibrotic M2 phenotypes and ST2/IL-33 signaling also contribute to disease progression [11,12]. **Figure 1** [13] denotes a stepwise framework for preventing graft-versus-host disease (GVHD) post-transplant involves:

- **Checkpoint 1 – T-cell depletion or inactivation**: Accomplished via ex vivo techniques or pharmacologic agents like anti-thymocyte globulin (ATG), post-transplant cyclophosphamide (PTCy), or alemtuzumab.
- **Checkpoint 2 – Inhibition of T-cell trafficking**: Strategies include blocking chemokine receptors like CCR5 (e.g., maraviroc).
- **Checkpoint 3 – Cytokine modulation**: Anti-inflammatory agents such as tocilizumab (anti-IL-6R) and JAK inhibitors (e.g., ruxolitinib) are used to reduce downstream immune activation.
- **Emerging checkpoint – Microbiome modulation**: Targeted antibiotics, probiotics, and fecal microbiota transplantation aim to restore gut homeostasis and Treg populations.

Approaches to prevent and treat GVHD after allogeneic stem cell transplantation –

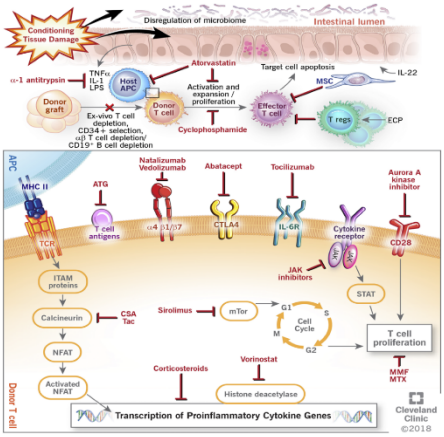

Figure 1 denotes a stepwise framework for preventing graft-versus-host disease (GVHD) post-transplant involves:

3. PREVENTION OF GRAFT-VERSUS-HOST DISEASE (GVHD)

Despite the availability of standardized prophylactic regimens, acute graft-versus-host disease (aGVHD) continues to affect approximately 20–50% of patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), posing a significant risk for non-relapse mortality (NRM) [14,15]. Chronic GVHD (cGVHD) manifests in nearly 30–40% of recipients, often leading to long-term complications and reduced quality of life, highlighting an ongoing need for more effective preventive approaches [16].

A fundamental aspect of GVHD prevention lies in donor selection. Transplants from HLA-identical sibling donors are associated with the lowest risk of both acute and chronic GVHD, establishing them as the preferred donor type [17]. In contrast, mismatches at HLA-A, -B, -C, and -DRB1 loci are strongly correlated with increased GVHD incidence and poorer survival outcomes [18]. Moreover, certain minor mismatches, such as those at HLA-DQB1 or non-permissive HLA-DPB1 alleles, may also elevate GVHD risk and are ideally avoided [19].

Recent insights by Ferrara and colleagues proposed a triphasic model for aGVHD prevention, targeting three critical checkpoints: (1) depletion or inactivation of alloreactive T cells, (2) inhibition of T-cell homing to target tissues, and (3) cytokine blockade to suppress inflammation [20]. More recently, the role of the gut microbiome in modulating GVHD pathogenesis has gained attention, especially concerning gastrointestinal involvement [21].

Immunoprophylaxis for GVHD primarily involves either direct T-cell modulation or the interruption of T-cell signaling. Common agents include anti-thymocyte globulin (ATG) and post-transplant cyclophosphamide (PTCy), both of which selectively deplete or inactivate donor-derived alloreactive T cells [22,23]. Another therapeutic axis involves targeting the T-cell receptor (TCR) pathway through calcineurin inhibitors (CNIs), which suppress IL-2 transcription and thereby inhibit T-cell activation and proliferation [24].

4. PHARMACOLOGICAL REGIMENS FOR GVHD PROPHYLAXIS

Standard prophylactic regimens typically combine a calcineurin inhibitor—either tacrolimus or cyclosporine—with methotrexate (MTX) or mycophenolate mofetil (MMF). MMF disrupts guanine nucleotide synthesis in T and B lymphocytes, thereby inhibiting cellular proliferation and

antibody production [25]. In comparative trials, tacrolimus combined with MTX was shown to be more effective than cyclosporine/MTX in reducing the incidence of grade II–IV aGVHD and severe cGVHD, although overall survival did not significantly differ [26,27].

A Swedish randomized controlled trial examining tacrolimus/MMF versus cyclosporine/MMF in patients receiving reduced-intensity conditioning found no significant differences in aGVHD incidence, relapse-free survival (RFS), or overall survival (OS) between the two arms [28]. Similarly, a Japanese trial comparing cyclosporine and tacrolimus in matched unrelated donor transplants reported comparable efficacy in preventing moderate to severe aGVHD [29].

Mycophenolate mofetil is particularly favored in regimens involving post-transplant cyclophosphamide or where MTX toxicity is a concern. Its use in combination with CNIs is now standard in haploidentical and matched unrelated donor (MUD) settings [30]. Table:1 summarizes the GVHD Prophylaxis regimens used in current settings.

Table 1: summarizing GVHD prophylaxis regimen

Regimen	Components	Mechanism of Action	Clinical Setting	Reference
CNI + MTX	Tacrolimus/Cyclosporine + MTX	IL-2 suppression, DNA synthesis inhibition	Matched sibling or unrelated donors	[13,14]
CNI + MMF	Tacrolimus/Cyclosporine + MMF	IL-2 inhibition + guanine synthesis blockade	RIC, CBT, mucositis-prone patients	[15]
PTCy-based	PTCy + CNI ± MMF	Selective deletion of alloreactive T cells, Treg preservation	Haploidentical and mismatched transplants	[28–30]
Sirolimus-based	Sirolimus + Tac ± MTX	mTOR inhibition, Treg sparing	High GVHD risk or MTX intolerance	[18,19]
ATG-based	ATG + CNI ± MTX/MMF	T-cell depletion, Treg preservation	MUD or MRD with MAC conditioning	[20–22]
Alemtuzumab-based	Alemtuzumab + CNI ± MTX	CD52-targeted lymphocyte depletion	UK centers, inborn error syndromes	[25–27]

CTLA4-Ig based	Abatacept + CNI + MTX	CD28-CD80/86 blockade, T-cell anergy induction	MUD and 7/8 matched donors	[31]
Maraviroc-based	Maraviroc + Standard Prophylaxis	CCR5 blockade, inhibits T-cell homing to liver/GI	Under trial for GI/liver GVHD prevention	[39]
Treg/Tcon Adoptive	CD34+ graft + Treg + Tcon infusion	Early immune modulation by Tregs	Haplo-HSCT with T-cell-depleted grafts	[35]
Microbiota-based	Antibiotics/Probiotics/FMT	Enhances Treg activity, restores microbial diversity	Emerging experimental therapy	[40,41]

5. ADVANCED IMMUNOSUPPRESSIVE STRATEGIES FOR GVHD PROPHYLAXIS

1. Sirolimus

The mammalian target of rapamycin (mTOR) inhibitor sirolimus has garnered attention for its dual ability to suppress effector T lymphocytes while preserving regulatory T cells (Tregs). Unlike calcineurin inhibitors (CNIs) such as tacrolimus, sirolimus has a distinct toxicity profile and is not associated with nephrotoxicity. In a randomized trial involving myeloablative conditioning with HLA-matched donors, sirolimus combined with tacrolimus was compared to the standard tacrolimus-methotrexate (MTX) regimen. While the incidence of grade II–IV aGVHD and cGVHD was comparable between groups, sirolimus appeared to offer protection against more severe (grade III–IV) aGVHD [31].

Further trials in the reduced-intensity conditioning (RIC) setting have also evaluated sirolimus-based regimens. A meta-analysis of over 1,600 patients indicated that sirolimus may modestly reduce the incidence of moderate aGVHD, though it did not significantly impact severe aGVHD, relapse, or overall survival. Notably, sirolimus was associated with a higher risk of transplant-related complications, such as thrombotic microangiopathy and veno-occlusive disease (VOD) [32].

2. Anti-Thymocyte Globulin (ATG)

Rabbit-derived anti-thymocyte globulin (ATG) serves as a potent immunosuppressive agent, capable of depleting host and donor T cells while sparing Tregs [33,34]. Multiple randomized controlled trials (RCTs) have demonstrated its efficacy in reducing the incidence of both aGVHD and cGVHD in patients receiving peripheral blood stem cell (PBSC) grafts from matched unrelated

or sibling donors. One study using a myeloablative regimen with tacrolimus and MTX showed a marked reduction in GVHD rates with the addition of ATG, although overall survival and non-relapse mortality outcomes were not significantly improved [35].

Comparative trials of different ATG formulations, such as Thymoglobulin versus Fresenius ATG, have also confirmed reduced cGVHD risk, but dosing, timing, and brand selection remain critical factors influencing outcomes [36,37].

3. Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody targeting the CD52 antigen expressed on B and T cells, offering broad lymphocyte depletion while sparing hematopoietic progenitor cells. In the UK, this agent has been incorporated into fludarabine-based regimens for both related and unrelated donor transplants, with encouraging reductions in GVHD incidence [38,39].

Recent trials have highlighted the importance of dose optimization. While earlier studies employed high-dose alemtuzumab, newer findings suggest that lower doses maintain efficacy while minimizing infectious complications. A randomized trial comparing a high-dose alemtuzumab/cyclosporine regimen to a triple-agent prophylaxis (tacrolimus, MTX, sirolimus) revealed superior control of chronic GVHD in the alemtuzumab group, albeit at the cost of increased relapse and infection rates [40].

4. Post-Transplant Cyclophosphamide (PTCy)

PTCy has revolutionized GVHD prevention in haploidentical transplants. It exerts selective cytotoxicity on proliferating alloreactive T cells while preserving Tregs and promoting immune tolerance through clonal deletion. Although PTCy does not completely eliminate donor T cells, it significantly reduces their proliferative capacity and pathogenicity [41].

Initially established in haploidentical settings, PTCy is now being evaluated in matched related and unrelated donor transplants as a potential replacement for traditional CNI-based regimens. Its efficacy in reducing GVHD risk without compromising engraftment or relapse control is under active investigation [42,43].

5. Abatacept

T-cell activation requires both antigen recognition via the T-cell receptor (TCR) and a co-stimulatory signal mediated by CD28 binding to B7 molecules (CD80/86) on antigen-presenting cells. Abatacept, a fusion protein (CTLA-4-Ig), blocks this second signal, thus preventing full T-cell activation. In clinical trials, abatacept was added to standard prophylaxis (CNI + MTX) in both matched and mismatched unrelated donor settings. The results demonstrated a significant reduction in aGVHD incidence, particularly in partially mismatched (7/8 HLA) transplants, without adversely affecting overall survival or relapse rates [44].

6. EMERGING STRATEGIES IN GVHD PROPHYLAXIS

Overview of Novel Regimens:

The exploration of innovative GVHD prophylactic methods is ongoing, aiming to minimize immunological complications while preserving graft-versus-leukemia (GVL) effects. Notably, grade II aGVHD has not been shown to predict long-term transplant failure, whereas grades III–IV GVHD are strongly associated with adverse outcomes including mortality and relapse. As such,

many recent clinical trials use composite endpoints such as GVHD-relapse-free survival (GRFS) and chronic GVHD-relapse-free survival (CRFS) to evaluate therapeutic success [45,46].

Large multicenter studies such as PROGRESS I and II have used these composite measures to compare the efficacy of different GVHD prophylactic regimens. These trials emphasize the importance of not only preventing GVHD but also maintaining disease remission and reducing immunosuppressive burden [47].

Targeted and Immune-Modulating Agents:

Several novel agents and strategies have demonstrated promising potential in early-phase trials:

a. **Tocilizumab:** an interleukin-6 receptor blocker, has been shown to significantly reduce GI tract aGVHD in phase 2 trials when added to tacrolimus and MTX. Only 14% of patients developed grade II–IV aGVHD, with a low incidence (3%) of severe disease [48].
b. **Abatacept:** which inhibits CD28-mediated co-stimulation, has shown promise in reducing early T-cell activation. In one small cohort, only 20% of patients developed moderate aGVHD, and no early transplant-related mortality was observed [49].
c. **Regulatory T-cell (Treg) Therapy:** involves the expansion and infusion of donor-derived or cord blood Tregs to restore immune tolerance. In a clinical study, umbilical cord-derived Treg infusions led to only 9% of patients experiencing grade II–IV aGVHD by day 100 [50].
d. **Ex Vivo T-cell Depletion:** (e.g., CD34+ selection or αβ T-cell removal) has also been explored as a method to reduce alloreactive T cells while preserving innate immune subsets like γδ T cells and NK cells. This technique showed reduced rates of both aGVHD and chronic GVHD [51].

Adjunct Strategies:

a. **Statins** have been investigated for their immunomodulatory effects, including the promotion of Treg expansion and inhibition of antigen-presenting cell activation. When used as adjuncts to tacrolimus and MTX, statins were associated with a remarkably low acute GVHD incidence (3.3%) [52].
b. **Vorinostat,** a histone deacetylase inhibitor, has demonstrated anti-inflammatory effects in early trials by dampening cytokine release and enhancing Treg function. When combined with tacrolimus and MMF, vorinostat reduced aGVHD to 22%, with severe disease in only 8% [53].
c. **JAK Inhibitors**, such as ruxolitinib and itacitinib, target inflammatory signaling pathways. These agents show preclinical promise and are under investigation for both prevention and treatment of GVHD while preserving anti-tumor immunity [54].
d. **Microbiome Modulation** through probiotics, antibiotics, or fecal microbiota transplantation (FMT) has emerged as a novel approach. Early evidence suggests that enhancing microbial diversity helps reduce inflammatory cytokines and boost Treg responses, particularly in the gastrointestinal tract [55,56]. Table:2 determines the current and investigational approaches for GVHD Prevention.

Table 2- Investigational Agents and Approaches for GVHD Prevention

Therapy	Mechanism of Action	Key Clinical Findings

Tocilizumab	Anti-IL-6 receptor monoclonal antibody	14% aGVHD (grade II–IV) by day 100 in phase 2 study [57]
Abatacept	Blocks CD28–CD80/86 costimulatory pathway	Reduced aGVHD in 7/8 HLA-mismatched transplant cohort [58]
Expanded Tregs	Restores tolerance, suppresses effector T cells	9% grade II–IV aGVHD with cord-derived Treg infusions [59]
CD34+ Selection	Removes αβ T cells, preserves NK and γδ T cells	Reduced chronic GVHD and improved immune reconstitution [60]
Statins	Enhances Tregs, inhibits Th1 and APC activation	Acute GVHD incidence of 3.3% in adjunct use [61]
Vorinostat	HDAC inhibitor, supports Tregs, reduces inflammatory cytokines	Grade III–IV aGVHD was <10% in RCTs [62]
JAK Inhibitors	Targets pro-inflammatory JAK/STAT signaling pathways	Under active trials for aGVHD prevention [63,64]
Microbiome Mod.	FMT or probiotics to restore gut flora and regulate Tregs	Early studies show promise in GI GVHD prevention [65,66]

7. FUTURE DIRECTIONS

Ongoing research is on tailored methods to GVHD prevention that retain immunological reconstitution and GVL activity. Janus kinase (JAK) inhibitors, such as ruxolitinib, are being used in novel techniques to target cytokine signaling pathways key to GVHD inflammation. Treg treatments, which involve ex vivo expansion and adoptive transfer, have showed promise in restoring immunological tolerance and avoiding GVHD. Furthermore, gut microbiota manipulation with antibiotics, faecal microbiota transplantation (FMT), or probiotics is being researched as a way to maintain gastrointestinal integrity and control immune responses. Biologic medicines such as anti-IL-6 monoclonal antibodies (e.g., tocilizumab) and anti-CD26 inhibitors provide further immune-modulation options. Personalized prophylaxis using biomarkers including blood ST2, REG3α, and BAFF levels has the ability to customize medication to individual risk profiles. Furthermore, gene-editing methods and tailored T-cell treatments hold promise for reducing alloreactivity while preserving disease management.

8. CONCLUSION

In conclusion, GVHD remains a significant consequence of allo-HSCT, with both acute and chronic variants presenting unique problems. A better knowledge of its immune-pathogenesis has led to the creation of more effective preventive regimens. Traditional combinations of calcineurin inhibitors and methotrexate remain common, but emerging methods such as PTCy, ATG, and costimulatory blocking have increased the prophylactic arsenal. Future advancements will rely on tailored immunotherapies, microbiome manipulation, biomarker-driven personalization, and cellular treatments. Continued research and clinical trials will be critical to improving prophylaxis approaches and patient outcomes after transplantation.

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