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## Review Article

# Graft-versus-Host Disease Prophylaxis and Immune Reconstitution After Allogeneic Transplantation

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## Abstract

**Background:** Prophylaxis against graft-versus-host disease has changed more in the past decade than in the preceding three, and each regimen shapes immune recovery differently.

**Objective:** To review the randomised evidence for current prophylaxis regimens and what each does to immune reconstitution.

**Methods:** Narrative review of randomised trials, registry analyses and reconstitution studies in allogeneic transplantation.

**Findings:** Post-transplant cyclophosphamide raised one-year graft-versus-host-disease-free relapse-free survival from 34.9% to 52.7% (hazard ratio 0.64), while overall survival, relapse and transplantation-related death did not differ substantially. Anti-lymphocyte globulin halved chronic disease with identical survival, and a registry analysis of 5,209 matched-sibling transplants found the two regimens indistinguishable except in relapse.

**Conclusions:** The gains are in composite endpoints. Whether they represent better transplantation or better-scored transplantation is the open question.

**Keywords:** graft-versus-host disease; cyclophosphamide; immune reconstitution; allogeneic transplantation; prophylaxis

## Introduction

Allogeneic transplantation cures by an immune mechanism and injures by the same one. The donor graft attacks residual malignancy and, indiscriminately, the recipient's skin, gut and liver, and every prophylaxis regimen attempts to separate the two [1].

For three decades the standard was a calcineurin inhibitor with methotrexate, established when the combination proved superior to cyclosporine alone [2]. What displaced it originated in haploidentical transplantation, where high-dose cyclophosphamide after graft infusion made transplantation across a full haplotype barrier feasible [3], then worked as single-agent prophylaxis [4], producing outcomes equivalent to contemporaneous matched transplantation [5]; the approaches have been reviewed [6]. The mechanism is not simply deletion of alloreactive cells [7].

This review considers what the randomised evidence supports, and what each regimen costs in immune recovery.

## Materials and Methods

Records were retrieved from Crossref, OpenAlex, Europe PMC and publisher pages for the terms *graft-versus-host disease*, *post-transplant cyclophosphamide*, *anti-thymocyte globulin*, *ruxolitinib* and *immune reconstitution*. Priority was given to randomised trials and to registry analyses large enough to be informative. Every bibliographic record was confirmed at source, and quantitative values were taken from the source rather than from secondary citation.

## Post-transplant cyclophosphamide in matched donors

The trial that changed practice compared cyclophosphamide, tacrolimus and mycophenolate against tacrolimus and methotrexate in reduced-intensity matched transplantation. At one year, adjusted

graft-versus-host-disease-free relapse-free survival was 52.7% (95% CI 45.8–59.2) with the experimental regimen and 34.9% (95% CI 28.6–41.3) with the standard, hazard ratio 0.64 (95% CI 0.49–0.83,  $p = 0.001$ ), among 214 and 217 patients respectively [8]. Patient-reported outcomes and two-year results were published subsequently [9].

Read the same abstract to the end and the qualification appears: overall and disease-free survival, relapse, transplantation-related death and engraftment did not differ substantially between the groups. The improvement is in a composite that counts graft-versus-host disease as an event.

Supporting randomised evidence is consistent in shape. A phase II trial compared three regimens against tacrolimus and methotrexate [10], a Dutch trial found benefit for the cyclophosphamide arm [11], and an Australian myeloablative trial reported three-year survival free of graft-versus-host disease and relapse of 49.3% against 14.1%, hazard ratio 0.419, in 134 adults — with improved relapse-free but not overall survival [12]. A registry analysis of 5,209 matched related transplants found the approaches indistinguishable except in relapse, 26.2% against 32.8%, hazard ratio 0.78 ( $p = 0.003$ ) [13].

## Serotherapy

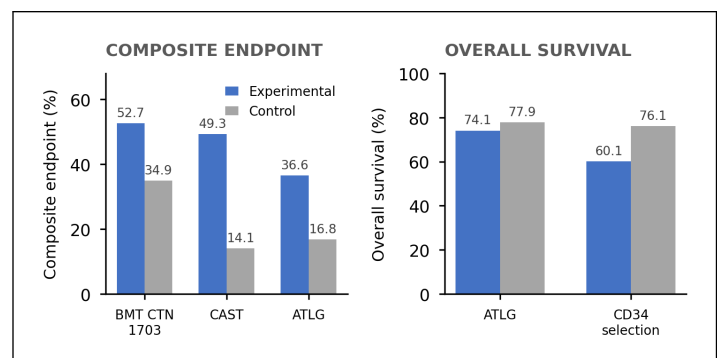


**Figure 1: Composite endpoint against overall survival in three randomised comparisons. Every regimen improves the composite; none improves survival, and CD34 selection worsens it. Data from Bolanos-Meade et al. [8], Kroeger et al. [16] and Luznik et al. [23].**

Anti-thymocyte and anti-lymphocyte globulin have been tested in randomised trials with a consistent result. Adding anti-T-cell globulin reduced chronic disease [14], as did pretreatment with anti-thymocyte globulin [15]. The clearest arithmetic comes from the anti-lymphocyte globulin trial: chronic disease at two years fell from 68.7% to 32.2%,  $p < 0.001$ , with the composite endpoint rising from 16.8% to 36.6%,  $p = 0.005$  — and two-year overall survival of 74.1% against 77.9%,  $p = 0.46$  [16]. A double-blind phase III trial assessed the same question [17].

Serotherapy has a dose-exposure relationship with immune recovery that is measurable and clinically relevant: anti-thymocyte globulin exposure is associated with CD4 reconstitution in paediatric transplantation [18], which is the mechanism by which it trades chronic disease against infection.

**Costimulation blockade and treatment of established disease**

Abatacept reduced severe acute disease, with day-180 severe-disease-free survival of 93.2% against 82% ( $p = 0.05$ ) randomised, and 97.7% against 58.7% in a mismatched cohort against registry controls [19].

For established steroid-refractory disease, ruxolitinib is now standard. A phase II study established activity [20]; the acute trial gave a day-28 response of 62% against 39% [21]; the chronic trial gave a week-24 response of 49.7% against 25.6% ( $p < 0.001$ ), with median failure-free survival exceeding 18.6 months against 5.7 [22].

T-cell depletion was tested against both alternatives. CD34 selection reduced moderate and severe chronic disease markedly, hazard ratio 0.25 (95% CI 0.12–0.52), yet two-year overall survival was 60.1% against 76.1%, hazard ratio 1.74 ( $p = 0.02$ ), driven by transplant-related mortality [23]. Here less chronic disease translated into worse survival.

**Immune reconstitution**

Recovery follows a stereotyped order — natural killer cells within 30 to 100 days, T cells by about 100 days, B cells over one to two years, with CD4 counts around 200/ $\mu$ l at three months [24]. Thymic output sets the pace and can be measured [25]; regeneration after immunological injury has been reviewed [26]. Graft source matters [27], and delayed reconstitution is associated with higher mortality [28].

Prophylaxis regimens shape this differently. Post-transplant cyclophosphamide impairs early T- and natural killer cell reconstitution [29]. A prospective comparison against anti-thymocyte globulin found lower day-30 counts with cyclophosphamide — total lymphocytes 473 against 924/ $\mu$ l ( $p = 0.0004$ ), T cells 82 against 238/ $\mu$ l ( $p = 0.022$ ), natural killer cells 96 against 385/ $\mu$ l ( $p < 0.001$ ) — while by day 180 B-cell recovery favoured it [30].

**Discussion**

Three conclusions follow. First, post-transplant cyclophosphamide improves a composite endpoint in matched-donor transplantation on good randomised evidence, and has not been shown to improve survival. Second, the same is true of serotherapy and, more starkly, of CD34 selection, where less chronic disease came with worse survival. Third, the regimens differ substantially in early immune recovery, which is the plausible route by which a reduction in graft-versus-host disease fails to become a reduction in mortality.

| Table 1: Randomised prophylaxis trials: the composite endpoint against overall survival. |                     |                               |                            |
|------------------------------------------------------------------------------------------|---------------------|-------------------------------|----------------------------|
| Trial                                                                                    | Comparison          | Composite endpoint            | Overall survival           |
| BMT CTN 1703 [8]                                                                         | PTCy vs Tac/MTX     | GRFS 52.7% vs 34.9%; HR 0.64  | No substantial difference  |
| CAST [12]                                                                                | PTCy/CsA vs CsA/MTX | GRFS 49.3% vs 14.1%; HR 0.419 | Not improved               |
| ATLG [16]                                                                                | ATLG vs none        | 36.6% vs 16.8%; $p = 0.005$   | 74.1% vs 77.9%; $p = 0.46$ |

| Trial              | Comparison                | Composite endpoint            | Overall survival         |
|--------------------|---------------------------|-------------------------------|--------------------------|
| BMT CTN 1301 [23]  | CD34 selection vs Tac/MTX | cGVHD HR 0.25 (0.12-0.52)     | 60.1% vs 76.1%; HR 1.74  |
| EBMT registry [13] | PTCy vs rATG, n = 5,209   | GRFS 46.5% vs 42.7%; p = 0.32 | 68.5% vs 64.8%; p = 0.92 |

Whether the composite endpoint is a legitimate surrogate or a construct that rewards one arm of the trade-off while concealing its infectious and relapse cost is the field’s genuine disagreement, and it is not resolved by any trial published so far.

This is a narrative review without systematic search or risk-of-bias assessment. Several quantitative results could not be confirmed at source and are therefore not reported, and trials differ in conditioning intensity, donor type and graft source in ways that prevent pooling.

Conclusion

Post-transplant cyclophosphamide has moved from a haploidentical technique to a matched-donor standard on the strength of randomised evidence for a composite endpoint, without a demonstrated survival benefit. Serotherapy shows the same pattern and CD34 selection the inverse one. The regimens are not interchangeable from the recipient’s point of view: they produce measurably different early lymphocyte recovery, and reconstitution kinetics are themselves associated with mortality. Trials should report immune reconstitution alongside disease incidence, and should present the components of any composite endpoint separately, so that a reduction in graft-versus-host disease can be weighed against what it costs.

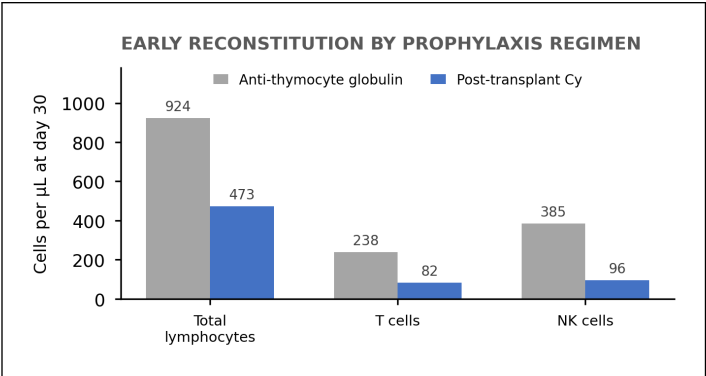


Figure 2: Lymphocyte subsets at day 30 after unrelated-donor transplantation with post-transplant cyclophosphamide against anti-thymocyte globulin. The delay is substantial and is the plausible route by which less graft-versus-host disease fails to become better survival. Data from Kerbaux et al. [30].

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Conflict of Interest

The authors declare that they have no competing interests relevant to the content of this review.

Ethics Statement

This article reviews previously published studies and contains no new studies with human participants or animals performed by any of the authors. Ethical approval was therefore not required.

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