HHS Public Access

Author manuscript

Transplant Cell Ther. Author manuscript; available in PMC 2022 June 01.

Published in final edited form as:

Transplant Cell Ther. 2021 June ; 27(6): 452–466. doi:10.1016/j.jtct.2021.02.035.

National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2020 Etiology and Prevention Working Group Report

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Financial disclosure:

Conflict of interest statement: K.M.W.: none. Y.I.: advisory boards for Novartis, Janssen and Meiji Seika Pharma. A.I.: none. B.H.: advisory board for Syndax. J.K.: research support from Amgen, BMS, Clinigen, Equillium, Miltenyi Biotec, and Regeneron Pharmaceuticals; advisory boards for Cugene and Therakos; consulting income from Biolojic Design, EMD Serono, Equillium, Gentibio, Moderna and Nekonal Oncology. M.A.: consultant for Fate Therapeutics; research funding from Pharmacyclics, Kadmon and Syndax. I.P.: advisory boards for Incyte, Kadmon and Syndax. J.W.M.: none. P.A.C.: consultant for Fate Therapeutics. L.L.: Receives research support from Genentech and Merck, serves on the advisory board: AbbVie, Talaris Therapeutics, PrecisionBiosciences and is a patent holder for WindMIL Therapeutics. P.R.: none. J.R.: research funding from Amgen, Equillium and Kite Pharma; Data Monitoring Committee for Avrobio; consulting income from BMS/Celgene, Infinity Pharmaceuticals, LifeVault Bio, Rheos Medicines, Talaris Therapeutics and TScan Therapeutics. H.G.: none. S.P.: Patent applications (US 20130115232A1 and WO2013066369A3) on "Methods of detection of graft versus host disease" licensed to ViaCore-IBT laboratories. B.R.B.: advisory boards for Magenta Therapeutics and BlueRock Therapeutics. Research funding from BlueRock Therapeutics. Steering committee: Kadmon Corporation. Co-founder Tmunity Therapeutics. J.P.: Consulting and advisory board membership – Syndax, CTI Biopharma, Amgen, Regeneron; Clinical trial support - Novartis, Amgen, Takeda, Janssen, Johnson and Johnson, Pharmacyclics, Abbvie, CTI Biopharma, BMS. C.C.: Consulting/Honoraria from: Incyte, Jazz, CareDx, Mesoblast, Syndax, Omeros, Pfizer. DW: advisory board Novartis and Incyte, DSMB: Novartis and Behring, honoraria from Takeda, Gilead, Pfizer and Neovii. K.R.S: DSMB member BMS/Juno, Advisory board Jazz, Novartis, Janssen. S.Z.P: none. S.J.L.: Research funding from Amgen, AstraZeneca, Incyte, Kadmon, Novartis, Pfizer, Syndax, Takeda. Steering committee: Incyte. P.J.M.: advisory boards for Mesoblast and Rigel Pharmaceuticals Inc.; honoraria from Janssen. G.S.: advisory boards for Novartis, Incyte, Pharmacyclics, Amgen, and Xenikos. S.S.: advisory board for Rigel Pharmaceuticals Inc.

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Abstract

Preventing chronic graft versus host disease (GVHD) remains challenging because the unique cellular and molecular pathways that incite chronic GVHD are poorly understood. One major point of intervention for potential prevention of chronic GVHD occurs at the time of transplantation when acute donor anti-recipient immune responses first set the events in motion that result in chronic GVHD. After transplantation, additional insults causing tissue injury can incite aberrant immune responses and loss of tolerance further contributing to chronic GVHD. Points of intervention are actively being identified so that chronic GVHD initiation pathways can be targeted without affecting immune function. The major objective in the field is to continue

basic studies and to translate what is learned about etiopathology to develop targeted prevention strategies that decrease the risk of morbid chronic GVHD without increasing the risks of cancer relapse or infection. Development of strategies to predict risk of developing debilitating or deadly chronic GVHD is a high research priority. This Working Group recommends further interrogation into mechanisms underpinning chronic GVHD development, and we highlight considerations for future trial design in prevention trials.

Introduction

An ideal method to prevent chronic graft versus host disease (GVHD) after allogeneic hematopoietic cell transplantation (HCT) would be to remove the donor cells that contribute to the risk of chronic GVHD from the graft or allow them to attain immune tolerance of recipient alloantigens that cause chronic GVHD, such that systemic immunosuppression is no longer necessary.[1] Recent studies have demonstrated substantial progress in preventing chronic GVHD using strategies that target immune cells with disease-inciting capacity, but prevention has not been fully realized because the underlying mechanisms that initiate chronic GVHD are only partially known. Identification of these mechanisms in human chronic GVHD in the early peri-transplant period remains challenging in part because a minimum of one year follow-up after HCT is needed to assess the chronic GVHD endpoint.

The advent of clinically relevant murine models and innovative measures of immune responses in mice and humans has helped identify the mechanisms that incite chronic GVHD.[2, 3] Mechanistic information from murine models, clinical trials and patient samples strongly suggests that pivotal chronic GVHD-inciting events involve certain donor T, B, monocyte, machrophage, innate populations such as ILCs and NK cells, and plasmacytoid dendritic cell subsets, donor and recipient antigen-presenting cells and recipient fibroblastic reticular cells. Chronic GVHD may be prevented by removing certain activated donor lymphoid populations, albeit with immune consequences. The extent of overlap between the antigens that trigger chronic GVHD, graft-versus-tumor (GVT) activity and pathogen defenses is not known. Further, while pathogens are typically cleared through activation of naïve cells that mature to effectors, the recipient alloantigens that trigger chronic GVHD and GVT activity generally persist. The challenge facing the field is to find ways to prevent chronic GVHD without increasing the risks of graft rejection, infection, and recurrent malignancy.

Purpose of this document:

This report reviews our current understanding of the etiologic factors that incite chronic GVHD, identifies knowledge gaps, and suggests cellular targets and mechanisms relevant to the benefits and risks in the design of clinical trials to prevent chronic GVHD. The problem of late acute GVHD is beyond the scope of this report. We distinguish between prevention, which is defined as interventions aimed at initiating or inciting events that occur after HCT but before any clinical or laboratory evidence of active chronic GVHD, and preemption, which is the topic of a future report.

Summary of recommendations:

Regarding the etiology and prevention of chronic GVHD, we reached consensus on the following points:

1. Moderate to severe chronic GVHD leads to excess long-term morbidity and mortality and should be prevented.
2. Chronic GVHD is initiated early after HCT and can also be incited late after HCT by additional insults.
3. Positive predictive values (PPV) for additional insults or secondary inciting events that occur after HCT are unknown, and observational studies should be performed to assess risk.
4. For patients with hematological malignancies, a risk assessment model should weigh both the risks of cancer relapse and graft rejection against the risk of severe chronic GVHD.
5. Studies of how damaged primary and secondary lymphoid organs contribute to altered T and B cell recovery and maturation in chronic GVHD genesis are needed.
6. Additional studies addressing manipulation of the donor graft and host tissue integrity and milieu will be informative.
7. We should study pediatric hosts and young donor stem cell products, including cord HCT, to define mechanisms of chronic GVHD etiology.
8. Targeting cells that we agree are not critical in GVT reactions, like certain B cell or T cell subsets or host stromal cell represent prime avenues for further clinical studies.
9. As the primary endpoint in prevention trials of chronic GVHD, especially in trials intended for regulatory review, we recommend survival without moderate to severe NIH-defined chronic GVHD (chronic GVHD-free survival).

Methods

Each working group was organized to encourage global engagement in the topic (see introduction to the series in this issue of *TCT*). Four groups worked individually beginning in February 2020 to review the relevant literature and prepare the initial draft of the manuscript. The Steering Committee reviewed and discussed the initial draft and offered recommendations for revisions. Two iterative rounds of comments and revisions were collected before the November 18–20, 2020 Consensus Conference. The manuscript was further revised for submission after additional suggestions from external reviewers, conference participants, and a 30-day public comment period.

I. Primary Insults – Immune cell-driven etiology of chronic GVHD and potential points of intervention

Current Knowledge: Donor, recipient, and exogenous factors contribute to chronic GVHD genesis, and dynamic interactions between these factors and secondary insults lead to immune dysregulation that manifests subsequently as chronic GVHD.[1] Studies in murine models[4, 5] and humans[6] indicate that events leading to chronic GVHD begin with tissue injury caused by the pretransplant conditioning regimen, which amplifies responses to alloantigens that trigger acute GVHD. Strategies that effectively reduce the risk of acute GVHD, however, have not necessarily reduced the risk of chronic GVHD and vice versa, highlighting the need for further elucidation of the mechanisms that determine these outcomes. Approximately 30% of patients develop chronic GVHD with no prior overt acute GVHD, either through a subacute graft-versus-host reaction or undefined independent mechanisms.[7] Considering that GVHD does not occur after autologous HCT, injury due to conditioning regimen alone is not sufficient for ongoing reactivity against recipient tissues. With current methods such as flow cytometry, it is difficult to distinguish the cells that cause chronic GVHD from those that prevent graft rejection, mediate GVT activity, and control infections. Better understanding of functional correlates and molecular drivers of immune cell subsets that mediate chronic GVHD is needed.

Gaps in knowledge and unmet needs; highest priorities: Figure 1 shows a working model of chronic GVHD inciting factors along with several potential points of clinical intervention that require further study. Clinical results suggest that the risk of chronic GVHD may be decreased by using aspirated marrow cells instead of growth factor-mobilized blood cells, using related donors, using younger donors, avoiding female donors for male patients, and using cord blood donors (Table 2).[8–12] Studies to determine whether the incidence of NIH chronic GVHD in adults is lower after CBT compared to PBSCT have yielded inconsistent results,[13, 14] but the severity of the disease among those who develop chronic GVHD is lower in cord blood recipients than in those who received mobilized blood cell grafts from HLA-mismatched unrelated donors.[15] Thus, donor selection serves as one point of intervention, which may become increasingly relevant as outcomes improve with alternative donor sources. Graft engineering before HCT and *in vivo* T cell depletion with anti-thymocyte globulin (ATG) or post-transplant cyclophosphamide (PTCy) remain areas of active investigation. Additional targets include B cells, monocytes, and macrhophages.

Donor T cells subsets: Pre-transplant graft engineering remains an area of active investigation in chronic GVHD prevention since conventional/effector alloreactive T cells (Tcon/Teff) are critical for initiating and mediating chronic GVHD. Evidence suggests that subclinical pathogenic processes begin long before the distinct clinical manifestations of chronic GVHD become apparent. Our understanding of human chronic GVHD etiology is largely based on data derived from *in vivo* and *ex vivo* graft manipulation trials to prevent acute GVHD.

Clinical studies demonstrate that higher donor T cell dose is a major risk factor for the development of chronic GVHD. The use of marrow as the stem cell source has an overall chronic GVHD incidence of approximately 30% while mobilized peripheral blood cell

products are associated with chronic GVHD in 50% of patients.[16] T cell depletion approaches have led to lower chronic GVHD rates of 10–40% for overall chronic GVHD and 10–20% for moderate and severe chronic GVHD.

In Table 1, we provide existing published data using *ex vivo* and *in vivo* approaches that have been linked to lower incidence and severity of chronic GVHD.[17–43] Approaches that reduce or deplete disease-initiating T cell populations may reduce chronic GVHD. Studies with CD34 selection, combined TCR alpha-beta$^+$ T cell and CD19$^+$ B cell depletion or naïve T cell depletion from the graft have been highly informative, although graft failure tended to be increased with some of these approaches. Many *in vivo* T-cell depletion studies have shown that the incidence and severity of chronic GVHD can be decreased by rabbit ATG, alemtuzumab or PTCy.[31–41] Potential risks associated with these interventions include graft failure, infections and relapse. The risks differ from one method to the next, requiring further evaluation.

Data in chronic GVHD and in immune-mediated diseases have identified functionally distinct subsets of proinflammatory Teff/Tcon cells that have pathogenic effects and anti-inflammatory regulatory T cells (Treg) that attenuate disease. Inducing apoptosis of rapidly dividing T cells early after HCT with methotrexate[44] attenuates the severity of acute GVHD but does not ameliorate chronic GVHD. Data also reveal that cord blood recipients have decreased incidence and severity of chronic GVHD also with relative increases in graft loss. Recipients of umbilical cord blood transplants may have a lower incidence or severity of chronic GVHD despite major HLA mismatching,[43, 45–47] although further studies are needed to evaluate the GVT activity of cord blood cells.[14, 45] Early phase clinical trials showed a low incidence of chronic GVHD after depletion of CD45RA-positive naïve T cells and B cells from mobilized blood cell grafts.[28] These data are consistent with results showing that both allogeneic donor T cells and recipient alloantigens are necessary to develop chronic GVHD in murine models.[48]

Emerging data suggest that PTCy may prevent GVHD not by deleting alloantigen-activated T cell subsets but by impairing their functions and possibly by increasing the numbers of Tregs.[49–51] As we further refine our understanding of the key T-cell subsets involved in chronic GVHD development and their phenotypic profiles, more selective approaches may be possible, for example, by targeting the pathogenic Th17 cells that have been linked to chronic GVHD.[52] Further research is needed to explore the full immunological impact of these acute GVHD prophylaxis methods to determine whether they have selective effects on specific cell populations that cause chronic GVHD.

The balance of Treg vs Teff/Tcon cells has a major role in the development of chronic GVHD. Treg/Teff ratios are low at the onset of chronic GVHD, and in patients without chronic GVHD, low Treg/Teff ratios may predict a high risk for chronic GVHD development.[53, 54] Adoptive transfer of *ex vivo*-expanded Treg or *in vivo* expansion mediated by low-dose IL-2 could decrease the risk of chronic GVHD through restoration of T cell immunoregulatory homeostasis.[55–58] Similarly, PTCy spares Treg.[50] Adoptive transfer or *in vivo* expansion of invariant natural killer T cells (NKT) may offer benefit, since the low numbers of these cells have been associated with low numbers of Tregs,

aberrant immune recovery and an increased risk of chronic GVHD.[59–61] Taken together, clinical evidence has led to an improved understanding of chronic GVHD. We have learned that targeting T, and possibly B cells, at the time of transplantation prevents chronic GVHD development and severity.

Donor B cell subsets: The presence of allo- and autoantibodies in patients with chronic GVHD suggests a pathogenic role for B cells, and results from murine models have shown that antibody-producing B cells contribute to the disease[62, 63] and are necessary in some models.[63–66] How antibodies mediate chronic GVHD is an area of active investigation. Proposed mechanisms include damage caused by antibody binding to thymic epithelial cell antigens[65] or antibodies to tissues.[63] B cells also act as antigen presenting cells after HCT.[67] The efficacy of B cell-depleting agents such as rituximab for treatment of chronic GVHD in some patients further suggests that pathogenic B cells contribute to clinical chronic GVHD.[68–70]

Two prophylaxis studies have suggested that *in vivo* depletion of $CD20^+$ B cells at 2–3 months after HCT may decrease the risk of chronic GVHD.[42, 71] Global depletion of $CD20^+$ B cells can induce prolonged B lymphopenia in mice and in patients. In some patients, eliminating $CD20^+$ B cells paradoxically has resulted in progression of chronic GVHD associated with delayed B cell reconstitution and high concentrations of the survival factor, B cell Activating Factor of the TNF family (BAFF), that support rare aberrantly activated B cells.[42, 60, 72–76] B cells that incite chronic GVHD are activated and primed for survival *in vivo*, and BAFF and alloantigen promote survival and B cell receptor (BCR)-activation of B cells in mice that develop chronic GVHD.[77] Potentially pathologic B cells have a lowered BCR signaling threshold that enables hyper-reactivity.[78, 79] These results have refined our understanding of these potentially pathogenic B cells and suggest that CD20 may not be the optimal target.[75] Selective depletion of constitutively activated, alloreactive B cells could be a more effective strategy to reduce the incidence and severity of chronic GVHD. This approach could attenuate chronic GVHD while allowing immune recovery of a comprehensive, diverse, peripheral B-cell compartment under physiologic homeostatic control.[79–81] Alternatively, low numbers of B regulatory cells (Breg) have been associated with aberrant immune recovery and chronic GVHD.[60, 61] These data suggest that identifying and implementing strategies to expand these cells *in vivo* could decrease the risk of chronic GVHD if unique cell surface markers can be identified that distinguish Bregs from plasmablasts.

Donor monocytes/macrophages/dendritic cells: The diverse mechanisms that contribute to T cell dysregulation in chronic GVHD include macrophages and dendritic cells, although their role in humans has been difficult to investigate. Studies of pulmonary chronic GVHD in murine models have suggested a significant role for donor-derived, alternatively activated macrophages (M2) that drive Th2- and Th17-cell activation[82] or via TGF-β secretion. Experimental models indicate that defective MHC class II antigen presentation during acute GVHD causes failure of Treg homeostasis in the periphery, thereby leading to chronic GVHD mediated by autoreactive T cells that escape negative selection in the thymus.[83] Murine data suggest that plasmacytoid dendritic cells are protective for chronic GVHD, and

there is some clinical data that may support this hypothesis as well.[84–87] Further research is needed to determine whether agents that selectively expand plasmacytoid dendritic cells *in vivo* could be used to prevent chronic GVHD in humans.[88]

Recipient fibroblastic reticular cells: Certain recipient stromal cells with immune functions in secondary lymphoid organs (SLO) have a role in the etiology of chronic GVHD. In mice, lymph node damage impairs T and B cell interactions through loss of fibroblastic reticular cells (FRCs) that are necessary to induce tolerance.[5, 89] FRCs, and potentially other recipient stromal cells, can also incite chronic GVHD via Notch ligand interactions that lead to aberrant activation of lymphocytes.[62, 90, 91] The high numbers of activated circulating follicular T and B cells in patients with chronic GVHD and the presence of circulating post-germinal center plasmablast-like cells that typically reside in SLO suggest SLO damage.[92] These CD4 T follicular helper (Tfh) cells interact with B cells, leading to increased plasma cell-like activation in active clinical chronic GVHD.[93] Whether the risk of chronic GVHD could be averted or diminished by optimizing the function of primary and secondary lymphoid organs is unknown, although the co-occurrence of immune recovery with restoration of primary and secondary lymphoid organ function and development of immune tolerance supports this possibility.[60, 61, 94]

Studies in murine models have shown that interactions between FRCs and B cells in recipient lymphoid organs may cause chronic GVHD.[62] FRCs are increased in number early after transplant and they have increased BAFF transcription.[77] FRCs may promote chronic GVHD because they are defective in their capacity to present recipient-tissue antigens and unable to mediate deletional tolerance.[5] Other myofibroblasts are pathologically activated in chronic GVHD[95] and may incite pathways leading to chronic GVHD. The strong association of chronic GVHD with poor recovery of TdT-positive and PAX5-positive cells in the marrow at day 30 after HCT[72] supports a need to move beyond cell surface phenotyping and enumeration of blood cells toward in-depth studies of interactions in recipient primary and secondary lymphoid organs and other tissues in animal models.

Roadmap to progress: Despite the many developments in the understanding of immune mediators of chronic GVHD, distinct mechanisms that incite disease remain elusive. Additional bench research is critical for the development of agents that will prevent disease. Understanding the etiopathology of chronic GVHD will also enable future risk stratification strategies.

II. Secondary Insults in chronic GVHD – Damage and dysfunction of recipient immune tissues and organs in chronic GVHD development and potential points of intervention

Current Knowledge: Loss of immune tolerance in patients with de novo autoimmune diseases is affected by age and infections. In HCT recipients, tissue damage from the conditioning likely induces chronic GVHD through antigen exposure and presentation, with damage propagated by infection, microbiome disruption, and loss of oral, pulmonary, and gastrointestinal mucosal integrity. The ability of target tissue to tolerate these types of injury likely varies between individuals, but further research to delineate mechanisms is needed.[96] Tissue signals drive recipient alloantigen presentation in murine models, inciting the

aberrant activation cascade that leads to chronic GVHD.[4] Approaches to minimize tissue injury in the preparative regimen with agents such as hematopoietic stem progenitor cell depleting anti-c-kit antibody could mitigate this contribution to chronic GVHD.[97]

Gaps in knowledge and unmet needs; highest priorities

Recipient thymic epithelial cell dysfunction: Impaired thymopoiesis is associated with chronic GVHD.[98] The thymus incurs damage from the preparative regimen, immunosuppressive medications and donor T cells.[65] In murine models, thymic damage impairs negative selection by medullary epithelial cells, permitting autoreactive donor-derived recent thymic emigrant T cells to target recipient tissues and mediate chronic GVHD.[48] Autoimmune regulator (*AIRE*) gene dysfunction and loss of intrathymic group 3 innate lymphoid cells caused by acute GVHD contribute to failure of negative selection in the thymus.[99, 100] The extent to which this mechanism applies in human HCT is not known. In both mice and humans, initial T cell reconstitution after HCT is derived primarily from expansion of mature T cells in the graft that have a restricted TCR repertoire.[53, 101–105] These results suggest that lack of thymic recovery and failure to generate a naïve T cell repertoire could contribute to chronic GVHD-associated immune dysfunction, although this causal relationship in humans remains to be proven. The decreased risks of chronic GVHD in children compared to adults raise the question of whether the lower rates of thymic involution positions faster thymic recovery that could have a protective effect by maintaining effective negative selection and robust production of Treg cells, achieving immune tolerance through effective de novo T cell production and immunoregulatory homeostasis.[13, 106–108] Additionally, lower risks of chronic GVHD may relate to preferential use of marrow and cord blood products for grafting in children.

Experiments in murine models have supported a role for androgen withdrawal, IGF-1 and keratinocyte growth factor (KGF) in thymic recovery.[109–112] These results have motivated trials to test whether androgen suppression, IGF-1 supplementation and KGF can decrease the risk of chronic GVHD. Results with KGF have not been encouraging,[113] and analyses of results with other agents are underway.

Non-immune organ tissue damage and chronic GVHD development: Certain exogenous events can be considered second insults that incite chronic GVHD by damaging recipient tissues other than primary or secondary lymphoid organs. In damaged tissues, the release of damage-associated molecular patterns (DAMPs) can trigger a proinflammatory microenvironment that leads to presentation of alloantigens or neo-autoantigens and intracellular antigens that are normally sequestered from the immune system.[114] Tissue damage, related either to viral and other infections or to the anti-viral immune responses, may incite chronic GVHD. Decreased amounts of surfactant in the lung before HCT, likely due to injured epithelia, confers an increased risk of lung GVHD.[115, 116] Elevated collagen type V, a marker of alveolar epithelial injury, has also been linked to pulmonary chronic GVHD.[117–119] Collectively, these examples support studies of agents that promote tissue repair and decrease the impact of viral infections early after HCT.

Data regarding the association of dental hygiene and oral health with the risk of oral chronic GVHD are limited. Periodontal disease leads to both gingival inflammation and breakdown. In one study, oral microbiome changes were associated with oral chronic GVHD.[120–122] Likewise, metabolic changes associated with the loss of gastrointestinal microbial diversity may be linked to GVHD.[123, 124] These data support studies that address the interaction between specific tissues and the local microbiome on chronic GVHD development.[124]

Factors that incite sclerotic skin and connective tissue manifestations of chronic GVHD remain less well known. The association of sun exposure and local mechanical stress with focal cutaneous manifestations of chronic GVHD suggest that recipient tissue responses may contribute to development of the disease. Further understanding of mechanisms leading to these outcomes will potentially lead to improved understanding of inciting events in chronic GVHD.

Roadmap to progress: While the contribution of "additional insults" has been suggested in chronic GVHD, research is critical to understand both the biology underpinning of these events in preclinical models and in humans and to elucidate ways to prevent morbid forms such as bronchiolitis obliterans and sclerotic cutaneous chronic GVHD.

III. Based on what we know about chronic GVHD etiology, how might we assess risk of chronic GVHD development?

Balancing the risks of moderate to severe chronic GVHD versus the risks of graft rejection, delayed immune reconstitution, and recurrent or progressive malignancy poses a key issue in designing trials to prevent chronic GVHD. As described in Figure 2, important considerations balance the benefit and risks of interventions at each time point after HCT. While lower intensity of the conditioning regimen may decrease the magnitude of tissue damage that might trigger chronic GVHD, it could also increase the risk of relapse or graft rejection. Interventions that decrease the numbers, activation, or survival of donor T and other functional immune cells could decrease the risk of chronic GVHD but could also decrease their ability to prevent graft rejection and recurrent or progressive malignancy (Table 1). Highly intensive immunosuppressive regimens could decrease the risk of chronic GVHD but could also delay immune recovery, increase susceptibility to infections, and possibly increase the risk of recurrent or progressive malignancy. Although interventions to prevent chronic GVHD could increase the risks of graft rejection, delayed immune reconstitution and opportunistic infections, effects on the risk of recurrent or progressive malignancy or the loss of therapeutic response in non-malignant diseases pose the most significant consideration in trial design.

IV. How do we best consider risk of recurrent or progressive malignancy as we consider prevention of chronic GVHD?

Several large studies have shown potent GVT effects associated with the presence of chronic GVHD by NIH criteria and an increased risk of recurrent or progressive malignancy in patients who did not develop chronic GVHD.[125–127] Mild chronic GVHD has been associated with improved overall survival for patients with malignant diseases, while moderate to severe chronic GVHD has been associated with an increased risk of non-relapse

mortality.[128, 129] During the first 18 months after HCT, patients without chronic GVHD who continued immunosuppressive medications had the highest risk of relapse.[125] Beyond 18 months after HCT, patients who did not experience chronic GVHD showed the highest risk of relapse even though they had discontinued all immunosuppressive medications, but treatment with immunosuppressive medications had no effect on the risk of relapse in patients who experienced acute or chronic GVHD in this study.[125]

Some of the therapeutic interventions with the greatest effect in preventing chronic GVHD have been associated with higher relapse rates (Table 1). Limitations of these studies include a lack of data addressing the risk for relapse as indicated by the presence or absence of measurable residual disease (MRD) before transplantation, the intensity of the preparative regimen, and the intrinsic susceptibility of the malignant cells to immunologic control. GVT effects may differ according to disease type and disease status.[126, 130] When evaluating the potential success of an intervention to prevent chronic GVHD, it is imperative to consider risk stratification for recurrent or progressive malignancy in the eligibility criteria. As discussed below, the presence of MRD at the time of HCT is associated with an increased risk of relapse after HCT. One approach of great interest would be to test agents that could simultaneously target malignant cells and chronic GVHD.[131] Emerging data suggest that adoptive transfer of certain NK subsets[132] or invariant NKT[133] or gamma delta T cells[134] could minimize the risk of chronic GVHD while preserving GVT benefits. Future studies should focus on testing whether adoptive transfer of anti-tumor lymphocytes could mediate critical GVT activity when depletion of donor lymphoid cells is used to prevent chronic GVHD.

V. Critical questions and answers about chronic GVHD prevention trials

Enrollment in prevention trials is based on risk factors known before HCT, regardless of when the intervention is given, while enrollment in preemption trials is based on post-transplant events, signs, symptoms or biomarkers indicating that the risk of chronic GVHD is higher than was previously appreciated. The sections below discuss considerations for prevention trials. Considerations for preemption trials are discussed in a separate report by Working Group 2.

The selection of interventions and approaches to test for prevention of chronic GVHD should be based on an understanding of the underlying mechanisms that initiate the processes leading to development of chronic GVHD, along with consideration of possible off-target effects and the impact on immune reconstitution after HCT. The following sections address three critical questions that should be considered in the design of trials testing new approaches to prevent chronic GVHD.

1) What preventive agents and approaches are most promising?—The key considerations include the strength of the efficacy data for the chronic GVHD intervention approach versus its influence on other HCT outcomes, including prevention of graft rejection, infections and recurrent or progressive malignancy.

Approaches that prevent chronic GVHD without impairing immune function would be ideal. Alternatively, selecting agents that could simultaneously target tumors and dysregulated

alloreactivity might mitigate any effect on the risk of relapse or graft rejection. To understand the influence of an investigational product or approach on relapse, it will be crucial to document the underlying pretransplant risk of relapse as accurately as possible. In patients with acute leukemia, the presence of MRD at the time of HCT reflects not only the measurable residual disease burden but also sensitivity of the disease to prior therapies, thereby representing the single strongest predictor of relapse after HCT.[135–137] Most reports of chronic GVHD prevention trials have not included information about the presence or absence of MRD at the time of HCT (Table 1). Including this and other information such as the disease risk index (DRI) will be important to understand the impact of specific approaches on the risk of relapse.[130] It may be possible to refine graft engineering approaches as we begin to understand the mechanisms leading to chronic GVHD. Alternatively, modification of current graft engineering approaches might improve the risk/benefit ratio, for example, by targeting the dose of ATG to the absolute lymphocyte count[138] by adjusting the numbers of T cells in the graft. Additionally, the timing of *in vivo* cell depletion or expansion strategies and adoptive transfer strategies, or application of ways to improve outcomes with cord blood grafts may improve the risk/benefit ratio.

2) Who should be enrolled in chronic GVHD prevention trials?—Ideally, trial inclusion and exclusion criteria should enrich for patients at high risk of moderate to severe chronic GVHD and exclude those at high risk of graft failure, infections, and relapse. Prevention trials can provide benefit only for the unknown subset of patients who would otherwise develop moderate or severe chronic GVHD. This potential benefit should be carefully weighed against the likelihood that the study intervention could increase the risks of graft rejection, viral reactivation, delayed immune recovery and recurrent or progressive malignancy that apply to all participants (Figure 3). Selecting patients with non-malignant diseases would obviate the risk of relapse, but most patients have malignant diseases. As shown in Table 1, in the absence of measures to prevent chronic GVHD, 25–40% of patients with non-malignant diseases develop this complication, which has no associated GVT benefit in these patients.[139–143] Future eligibility criteria could include biomarkers that have a high positive predictive value for development of chronic GVHD. If the risk of a study intervention is low, enrollment of patients with a low risk of developing chronic GVHD may be justified but could require a larger sample size. If the risk of the study intervention is high, however, enrollment of patients with a low risk of developing chronic GVHD may not be justified. Developing prognostic models that quantitate these risk/benefit ratios based on existing data is a high research priority (Table 2).

3) What are the most appropriate endpoints in chronic GVHD prevention trials?

3.1) Primary efficacy endpoint: Supplementary Table 1 summarizes currently active interventional phase 2 or 3 trials with prevention of chronic GVHD as the primary endpoint from ClinicalTrials.gov. The primary endpoints in these trials vary in terminology, (e.g., “moderate/severe,” “extensive,” “requiring steroid treatment”), incorporation of events other than chronic GVHD (i.e., acute GVHD, recurrent malignancy, and death), specification of time points for assessment, and statistical methods for analysis (e.g., log-rank test, proportional hazards, Gray test).

To move the field toward pivotal chronic GVHD prevention trials intended for regulatory review, we recommend survival without moderate to severe NIH-defined chronic GVHD (chronic GVHD-free survival) as primary endpoint. In two studies that have reported this endpoint, the probabilities of moderate to severe chronic GVHD-free survival were 44% and 47% at 2 years in patients who received standard post-transplant immunosuppression with tacrolimus and methotrexate.[24, 41] Cumulative incidence estimates of moderate to severe chronic GVHD are not appropriate as the primary endpoint, because they can be decreased by a high incidence of death or relapse as competing risks. Chronic GVHD requiring systemic treatment could serve as a functional endpoint definition instead of moderate to severe chronic GVHD, although this endpoint depends on providers' medical judgment regarding the need for systemic treatment, which could be biased. We recommend assessing the primary endpoint at one year after HCT in prevention trials because most chronic GVHD develops within one year, but longer follow-up beyond the primary endpoint at one year would be highly desirable.

In earlier phase studies, an endpoint that captures an effect linked to chronic GVHD prevention would be appropriate as a primary endpoint. For example, if an intervention is intended to increase the number of Tregs or to improve their function as a way to prevent chronic GVHD, an early phase trial could be designed around these immunological endpoints, provided that the established linkage to prevention of chronic GVHD is strong.

3.2) Secondary endpoints: Composite endpoints such as chronic GVHD-free, relapse-free survival (CRFS) or GVHD-free, relapse-free survival (GRFS) have gained popularity as a way of assessing the overall success of HCT. Composite endpoints are most appropriate as secondary endpoints because the onset of failure events other than chronic GVHD (i.e., relapse, death, and severe acute GVHD) can confound the interpretation of the most relevant failure event (i.e., chronic GVHD). GRFS is appropriate if it is anticipated that the study intervention is likely to decrease the incidence of both acute and chronic GVHD. For chronic GVHD prevention studies, CRFS is preferred secondary endpoint over GRFS. In all studies, separate reporting of each component in composite endpoints and tabulation of the causes of death should be included as secondary endpoints to understand the benefits and risks of the study intervention and to understand how these events influence the interpretation of the primary endpoint. We encourage investigators to develop and validate patient-reported measures that could be used for periodic screening to detect the onset of chronic GVHD. Prevention trials also offer an opportunity to collect biospecimens that could be used in exploratory studies to identify biomarker changes that reliably predict the future development of chronic GVHD.[2]

3.3) Interpretation of results: Pivotal studies intended for regulatory review must be adequate and well controlled, generally by comparing results in the investigational arm versus randomized placebo concurrent controls, no treatment concurrent controls, active treatment concurrent controls or pre-specified historical controls (21CFR§314.126).

To gain efficiency, trials with more than 2 arms can be designed to compare multiple investigational arms against a single control arm. The major disadvantage of this approach is that large numbers of patients are needed to compare the investigational arms against each

other, making it difficult to complete them in a timely manner. Another disadvantage is that enrolled patients must be able to receive any of the study interventions, potentially excluding some patients who have contraindications to only one of the study arms. Single arm designs may be appropriate for phase 2 studies, but the interpretation of results is currently limited by the lack of validated risk stratification criteria and benchmarks for the probability of survival without moderate to severe chronic GVHD. Therefore, randomization may be preferred for phase 2 studies, if feasible. Participants in trials to prevent chronic GVHD should generally not co-enroll in trials to prevent other major complications such as relapse, unless stratification is used to balance the study arms in a randomized trial.

The most appropriate control arm in chronic GVHD prevention trials is the standard of care when the trial is designed. Retrospective and prospective studies are needed to develop pre-transplant risk stratification for the incidence of moderate to severe chronic GVHD with standard of care treatment regimens, thereby informing the eligibility criteria for future trials. Studies are also needed to provide benchmarks for the probability of survival without moderate to severe chronic GVHD, thereby informing the design and interpretation of results in future trials.

Roadmap to progress: Clinical trials designed to prevent moderate to severe chronic GVHD should include a risk assessment model for relapse in patients with underlying malignancy with careful consideration of the eligibility criteria for these patients. Investigators should also consider the competing risks of graft failure and infections in those with nonmalignant indications. We recommend survival without moderate to severe NIH-defined chronic GVHD (chronic GVHD-free survival) as the primary endpoint in prevention trials of chronic GVHD.

Below we summarize recommendations for studies over the next 3 years

1. Elucidate the distinct cellular and molecular pathways that induce and maintain immune tolerance, thereby allowing withdrawal of immunosuppressive medications without risk of clinically evident recurrent or progressive chronic GVHD.
    - **1.a.** Use primary patient samples and murine models to determine how recipient and donor characteristics incite or attenuate the development of chronic GVHD.
    - **1.b.** Define roles for recipient tissues and organs in the initiation of chronic GVHD.
    - **1.c.** Determine how additional insults after HCT incite moderate to severe chronic GVHD.
2. Integrate studies of patient samples to include potential use of high throughput “multi-omic” approaches and systems immunology approaches to identify predictive risk assessment markers.
3. Work toward defining a risk stratification strategy that predicts risks of relapse, graft failure, and viral infections, and balances these against the risk of

developing morbid forms of chronic GVHD to identify patients who would most benefit from chronic GVHD prevention trials.

4. Conduct well-designed chronic GVHD prevention trials based on what we know about the balance of benefits and risks to optimize immune reconstitution without impairing GVT activity.

We conclude that well-designed trials to prevent chronic GVHD would best be informed by basic and translational discoveries that have originated from clinical observations. A collaborative bedside-to-bench-to-bedside approach with careful considerations of the risk-benefit ratio regarding diminish morbidity without increasing mortality is needed to prevent chronic GVHD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS:

Funding and implementation of this Consensus Project is made possible through the support by the Intramural Program of the National Cancer Institute – Center for Cancer Research and the National Institute of Dental and Craniofacial Research, the NIH Intramural and Extramural Research Programs Institutes and Centers. Special acknowledgement goes to the Meredith Cowden GVHD foundation, France Lymphome Espoir, NBMTLink; Anthony Nolan, National Marrow Donor Program, BMT InfoNet and other patient advocacy groups for partnering and collaboration. Thanks go to all working groups and consensus conference participants, professional societies, US government agencies and stakeholders in the field of hematopoietic stem cell transplantation for the generous donation of their work, time, talents and expertise. Particular acknowledgment to the ASTCT and EBMT for their roles in the dissemination, education and implementation of the concepts and best practices evolving from this project.

Special thanks to the independent external peer reviewers who provided they comments and critiques to the 2020 NIH Chronic GVHD Consensus Project: **Nicolaus Kroeger, M.D.** Professor & Clinical Director of the Department of Stem Cell Transplantation, University of Hamburg, Hamburg, Germany, President EBMT; **Ryotaro Nakamura, M.D.** Hematologist – Oncologist, Professor & Director of the Center for Stem Cell Transplantation City of Hope Cancer Center, Duarte, California; **John DiPersio, M.D., Ph.D.** Chief, Division of Oncology; Director, Center for Gene and Cellular Immunotherapy; Deputy Director, Siteman Cancer Center, Washington University School of Medicine, St. Louis, Missouri; **Mark Juckett, M.D.,** Professor & Director of the Blood and Marrow Transplant Program, University of Wisconsin, Madison, Wisconsin; **George Chen, M.D.,** Associate Professor of Medicine, University at Buffalo, Buffalo, New York; **Rafael Duarte, M.D., Ph.D., FRCP,** Head of Department of Hematology and Director of the Hematopoietic Transplant Program, Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain; **Franco Locatelli, M.D.** Professor of Pediatrics Università Sapienza, Roma, Head of the Department of Pediatric Hematology and Cell and Gene Therapy, IRCCS Ospedale Pediatrico Bambino Gesù, Roma, Italy; **Areej El-Jawahri, M.D.,** Assistant Professor, Director of the Bone Marrow Transplant Survivorship Program, and Associate Director of the Cancer Outcomes Research and Education Program at Massachusetts General Hospital, Boston, Massachusetts; **Robert Soiffer, M.D.** Professor & Co-Chief of Stem Cell Transplantation and Cellular Therapies, Chair of the Executive Committee for Clinical Programs, Vice Chair for the Department of Medical Oncology, Chief of the Division of Hematologic Malignancies, Dana Farber Cancer Institute, Boston, Massachusetts; **Daniel Weisdorf, M.D.,** Professor of Medicine & Deputy Director of Clinical Science and Translational Science Institute; Director, Clinical and Translational Research Services, University of Minnesota, Minneapolis, Minnesota; **Keith Sullivan. M.D.,** Professor of Medicine, Hematologic Malignancies and Cellular Therapy, Duke University Medical Center, Durham, North Carolina; **Catherine Lee, M.D.,** Assistant Professor, Hematology and Medical Oncology, Huntsman Cancer Institute - University of Utah, Salt Lake City, Utah; **Jose Antonio Perez-Simon, M.D.,** Professor of Hematology, University of Seville, Head of Department of Hematology, University Hospital Virgen del Rocio and Vice director of the Biomedical Research Institute of Seville (IBIS), Seville, Spain; **Doris Ponce, M.D.,** Associate Professor of Medicine, Hematologic Oncologist, Memorial Sloan-Kettering Cancer Center, New York City, New York; **Andrew Harris, M.D.,** Pediatric Hematologist-Oncologist & Assistant Professor of Pediatrics, Pediatric BMT and Cellular Therapy Program, University of Utah/Primary Children's Hospital, Salt Lake City, Utah.

APPENDIX:: 2020 NATIONAL INSTITUTES OF HEALTH CONSENSUS-DEVELOPMENT PROJECT ON CRITERIA FOR CLINICAL TRIALS IN CHRONIC GVHD STEERING COMMITTEE

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Consensus Key Point:

Moderate to severe chronic GVHD leads to excess morbidity and mortality and should be prevented.

- Despite the advent of effective chronic GVHD prevention strategies, further scientific and clinical research is needed.
- T-cell depletion strategies decrease the risk of chronic GVHD but can also impair immune reconstitution and anti-tumor effects after HCT.

Consensus Key Point:

Primary inciting cellular and molecular pathways leading to chronic GVHD arise from donor and host factors and are triggered early after HCT.

Points of Intervention – Mitigation of Risk Factors for chronic GVHD Development

- Graft engineering strategies
- Modified schedules for weaning immunosuppression (IS) after HCT
- Protection of primary and secondary lymphoid organs (SLO)
- Maintaining balance between immune effector cells and immune regulatory cells

Consensus Key Point:

Agents that target cells that might not be critical in GVT reactions, such as certain T cell subsets, distinct B cell subsets, monocytes, macrophages, or host lymphoid organ stromal cells represent prime candidates for further clinical studies.

Points of intervention to mitigate chronic GVHD development

- Small molecule inhibitors of aberrant B and T cells
- Antibodies to cytokines

Key Consensus Point:

Secondary insults may occur at any time after HCT.

Develop Risk Mitigation Strategies based on second insults leading to chronic GVHD

- HLA matching
- Infection prevention
- Tissue damage prevention

Consensus Key Point:

For patients with hematological malignancies, a risk assessment model for moderate to severe chronic GVHD must consider possible effects of the study intervention on the risk of recurrent or progressive malignancy when designing clinical trials.

Points of intervention to mitigate chronic GVHD development

- Develop risk stratification tools to guide clinical trial design
- Consider interventions that serve dual purposes by targeting both chronic GVHD and malignant cells in the recipient

Consensus Key Point:

Integrate studies of patient samples, to include potential use of high throughput “multi-omic” approaches in a systems immunology approach to aid in chronic GVHD risk stratification

Points of intervention to mitigate chronic GVHD development

- Use translational data to identify populations at high risk of chronic GVHD
- Use translational data to identify novel targets

Consensus Key Point:

Despite the advent of effective chronic GVHD prevention strategies, further clinical trials are needed.

- We recommend moderate to severe chronic GVHD-free survival as the primary endpoint in trials to prevent chronic GVHD.

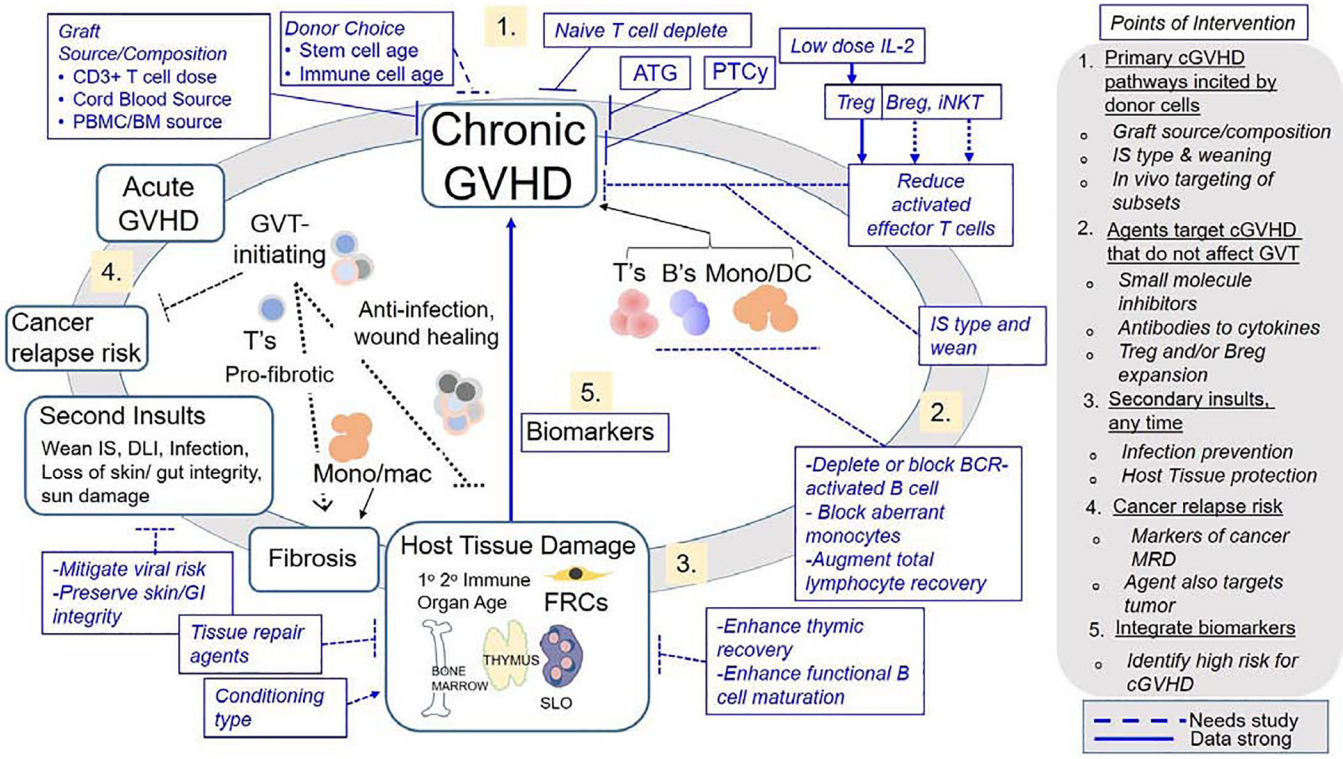

Figure 1. The etiology of chronic GVHD and the potential points of clinical intervention.

Recipient factors include age, damage to bone marrow stroma, thymus, secondary lymphoid organs (SLO) (i.e., spleen, lymph nodes and other lymphoid tissues), and fibroblastic reticular cells (FRCs). The choice of agents in conditioning regimens and the overall intensity of conditioning regimens influences the extent of damage to these organs. Less robust evidence suggests a role for recovery of these organs in prevention of chronic GVHD, including thymus recovery, functional B cell maturation, and tissue repair. **Donor graft factors** include donor age, $CD3^+$ T cell dose, and graft source. Donor cell products contain heterogenous cell populations that contribute to acute GVHD, chronic GVHD, graft-versus-tumor activity, pathogen defense and tissue repair. **Donor graft points of intervention** include non-selective T cell depletion, selective depletion of naïve T cells and other graft engineering. Post-transplant cyclophosphamide (PTCy) may silence alloantigen-activated T cells or induce alloreactive T-cell functional impairment while sparing Treg cells, while low-dose IL-2 could expand Treg. Other graft engineering approaches could target induction of Breg and iNKT cells. Secondary insults occur after infusion of HCT and include withdrawal of immunosuppression, donor lymphocyte infusion, infections, loss of gastrointestinal integrity, and ultraviolet damage to the skin. Potential points of clinical intervention are shown in blue font inside blue boxes. Arrows and block symbols depicted with solid lines indicate strong evidence, while dashed arrows and block symbols represent less robust evidence.

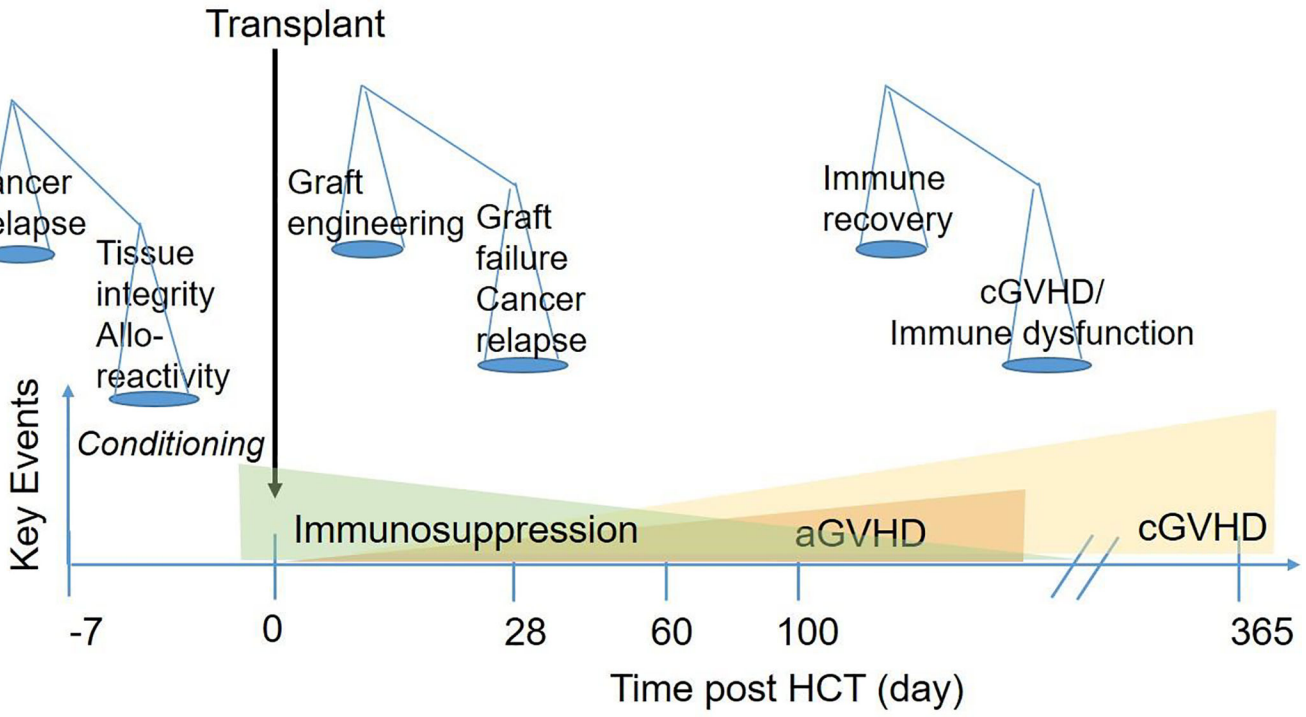

Figure 2. Factors that influence the emergence of chronic GVHD.

The x-axis shows time after HCT, with key events denoted in shapes. The green triangle indicates gradual tapering of immunosuppressive treatment after HCT. The orange triangle denotes the onset of acute GVHD, and the yellow triangle denotes the onset of chronic GVHD, both of which can be masked by immunosuppressive treatment. High-intensity pre-transplant conditioning regimens can decrease the risk of relapse but increase the risk of chronic GVHD. Depletion of donor T cells can decrease the risk of chronic GVHD but increase the risks of graft rejection, infections due to delayed immune reconstitution and relapse due to loss of GVT activity. Withdrawal of immunosuppression permits immune recovery and protection against infections but can increase the risk of chronic GVHD.

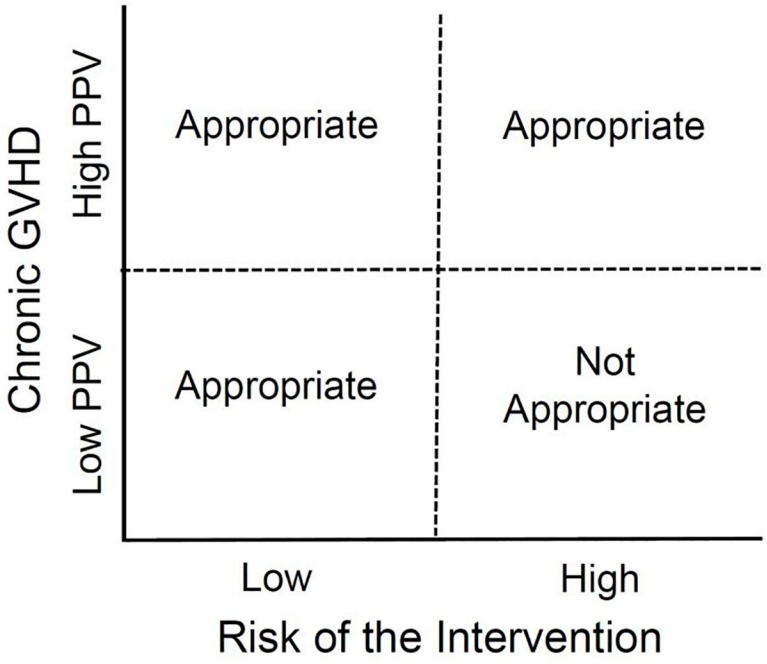

Figure 3. Critical considerations of risk and benefit in the design of chronic GVHD prevention trials.

The positive-predictive value (PPV) of a prognostic measure or algorithm before HCT estimates the probability of developing chronic GVHD in patients who have a positive test result. The dashed lines represent hypothetical boundaries between the low and high-risk interventions (X-axis) and between low and high PPV (Y-axis). Patients not destined to develop chronic GVHD cannot benefit in prevention trials. Therefore, clinical trials should be designed to ensure that the harm of the intervention in these patients does exceed the benefit in patients destined to develop chronic GVHD.

Table 1.

Clinical studies that have informed us about potential early points of intervention and the etiology of chronic GVHD

Approach	In vivo/ ex vivo	Study design	Cells targeted	Backbone GVHD ppx	Cohort	%MRD positive	Donor	Cell source (n)	%Moderate to severe* chronic GVHD	%Any chronic GVHD	%Relapse	%Graft failure	%OS
Anti-CD19 Alpha/β	Ex vivo	Ph1/2	Alpha/β T, B	ATG pre + rituximab	Ped/HM/ TBI	N/A	Haplo	PB (81)	0	0	24	2	71
Anti-CD19 Alpha/β	Ex vivo	Pilot	Alpha/β T, B	ATG pre + CNI ± MTX	Ped/HM/ TBI	18	Haplo/M UD	PB (33)	12	30	30	0	67
Anti-CD19 Alpha/β	Ex vivo	Retro	Alpha/β T, B	ATG pre	Ped/NM	N/A	Haplo	PB (14)	21	N/A	N/A	14	84
Anti-CD19 Alpha/β	Ex vivo	Ph1/2	Alpha/β T, B	ATG pre + rituximab	Ped/NM	N/A	Haplo	PB (23)	0	0	N/A	17	91
ATG vs. None	In vivo	Ph3	ATG rabbit	CNI +MTX	Adult/H M	N/A	MUD/ MSD	PB (155)	6 vs. 33	27 vs. 64	32 vs. 26	0 vs. 1	74 vs. 78
ATG vs. None	In vivo	Ph3	ATG rabbit	CNI +MTX	Adult/H M	N/A	MUD	PB (164)/ BM (37)	12 vs. 45	30 vs. 60	33 vs. 28	N/A.	55 vs. 43
ATG vs. None	In vivo	Ph3	ATG rabbit	CNI +MTX	Adult/H M	N/A	MUD	PB (196)/ BM (49)	12 vs. 33	16 vs.38	32 vs. 21	21 vs. 6	59 vs. 74
ATG vs. None	In vivo	Ph3	ATG rabbit	CNI +MTX or MMF	Adult/H M	N/A	MUD/ MMUD	PB (173)/ BM (23)	13 vs. 29	22 vs. 33	11 vs. 16	3 vs. 2	74 vs. 79 @6 mo.
ATG vs. None	In vivo	Ph3	ATG rabbit	CSP +MTX +MMF	Adult/H M	N/A	MSD	BM +PB (101)/ PB (153)/ BM (9)	8.5 vs. 23	28 vs. 53	21 vs. 15	0 vs. 0	69 vs. 70
AntiCD45RA + CD34 selec.	Ex vivo	Ph2	Naive T/ CD34−	TAC	Adult/H M/TBI	37	MSD	PB (35)	3	9	21	0	78
CD34 selec.	Ex vivo	Ph2	CD34−	None	Adult/H M/TBI	N/A	MSD	PB (44)	7	18	24	0	60
CD34 selec.	Ex vivo	Retro	CD34−	ATG	Adult/H M/TBI	N/A	MSD≥7/8	PB (241)	1	5	22	<1	57
PTCy	In vivo	Ph2	Activated T	CSP	Adult/H M	49	MSD/MU D	PB (45)	30	N/A	17	2	70
PTCy	In vivo	Retro	Activated T	CNI +MMF	Adult/H M	58	Haplo	BM (104)	N/A	30	44	10	45
PTCy	In vivo	Retro	Activated T	None	Adult/H M	58	MSD/MU D	BM (297)	N/A	12	37	5	72
PTCy	In vivo	Ph2	Activated T	None	Adult/H M	47	MSD/MU D	BM (92)	14	14	22	5	67

Approach	In vivo/ ex vivo	Study design	Cells targeted	Backbone GVHD ppx	Cohort	%MRD positive	Donor	Cell source (n)	%Moderate to severe* chronic GVHD	%Any chronic GVHD	%Relapse	%Graft failure	%OS
PTCy	In vivo	Retro	Activated T	CNI +MMF	Adult/HM	34	MUD	BM (150)	15	45	24	8	57
PTCy	In vivo	Retro	Activated T	None vs. CNI +MTX	Ped/HM/ TBI	45 vs. 22	MSD	BM (29)	0 vs. 0	0 vs. 6	45 vs. 44	0 vs. 0	54 vs. 58
PTCy	In vivo	Retro	Activated T	None	Ped/NM	N/A	Haplo	BM (27)	0	24	N/A	22	78
PTCy	In vivo	Ph2	Activated T	CNI +MMF	Both/HM	N/A.	Haplo	BM (68)	13	N/A	51	13	26
PTCy	In vivo	Ph1/2	Activated T	None	Adult/HM	N/A.	MSD/MUD	BM (117)	3	10	44	3	55
PTCy+MMF vs. BOR +MTX vs. MVC+MTX	In vivo	Ph2	Activated T	TAC	Adult/HM	N/A.	MSD/ MUD≥7/8	PB (273)	22 vs. 29 vs. 33	28 vs. 39 vs. 43	28 vs. 24 vs. 31	4 vs. 6 vs. 4	71 vs. 68 vs. 66
PTCy vs. TAC+MTX vs. CD34 selec. +ATG	In vivo	Ph3	Activated T	None	Adult/HM	N/A.	MSD/MUD	BM (346)	27 vs. 34 vs. 9	42 vs. 44 vs. 23	14 vs. 26 vs. 21	0 vs. 1 vs. 3	76 vs. 76 vs. 60
Anti-CD20	In vivo	Ph2	B cells	N/A	Adult/HM/ RIC60%	46 vs. 41	MSD MUD	PB (65)	31 vs. 49	48 vs. 60	34 vs. 28	N/A	71 vs. 56
CB Treg vs. CB control		Ph1	Activated T	Siro +MMF	Adult/HM	N/A	MMUD Cord >3/6	CB (11)	N/A.	0 vs 14	33 vs. 40	9 vs. 14	81 vs. 61 @ 1 yr
CB vs. MUD		Retro		CNI +MTX or MMF	Both/HM	31–39	Cord >3/6 MUD	CB (140)/ PB (237)/ BM (107)	N/A but no. diff.	N/A but no. diff.	15 vs. 24	N/A	71 vs. 63
CB vs. MUD		Retro		CNI +MTX or MMF	Adult/HM/TBI	N/A	Cord >3/6 MUD	CB (116)/ PB (361)/ BM (185)	23 vs. 34	39 vs. 42	22 vs. 25	8 vs. 3	44 vs. 43
CB vs. MUD/MSD		Retro		CNI +MTX or MMF	Both/HM/TBI	Equiv.	Cord>3/6 MUD/MSD	CB (128)/ PF (275)/ BM (81)	N/A	26 vs. 43–47	15 vs. 37–43	10 vs 0	N/A, LFS: 51 vs.33–48
CB		Ph2		CNI +MMF	Adult/NM	N/A	Cord >3/6	CB (26)	12	36	N/A	12	85
PB vs. BM		Ph3		CNI +MTX	Adult/HM		MUD/ MMUD	PB (273)/ BM (278)	48 vs. 32	53 vs. 41	30 vs. 30	2 vs. 6	46 vs. 51
PB/BM/CB		Retro CIBMTR		All	Pediatric/ NM	N/A	All (20% MSD)	All (1696)	N/A	25	N/A	<1	75
PB/BM/CB		Retro CIBMTR		All	Adult/ pediatric Sickle cell	N/A	MMUD/ MUD/ Cord >3/6	All (352)	N/A	29–32	N/A	17–44	81–87

Approach	In vivo/ ex vivo	Study design	Cells targeted	Backbone GVHD ppx	Cohort	%MRD positive	Donor	Cell source (n)	%Moderate to severe* chronic GVHD	%Any chronic GVHD	%Relapse	%Graft failure	%OS
PB/BM		Retro CIBMTR		All	Adult/ pediatric SAA	N/A	MUD/ MMUD	BM (409)	N/A	28–40	N/A	8–15	71–80

*Some studies report extensive chronic GVHD.

MRD, measurable residual disease; PTCy, post-transplant cyclophosphamide; BOR, bortezomib; MVC, maraviroc; TAC, tacrolimus; CB, cord blood; MUD, HLA-matched unrelated donor; MSD, HLA-matched sibling donor; PB, peripheral blood stem cell; BM, bone marrow; ATG, anti-thymocyte globulin; CNI, calcineurin inhibitor; MTX, methotrexate; MMF, mycophenolate mofetil; CSP, cyclosporine; Siro, sirolimus; HM, hematological malignancy; NM, non-malignancy; N/A, not available; MMUD, HLA-mismatched unrelated donor.

Table 2.

Factors associated with an increased risk of chronic GVHD

Donor factors	G-CSF mobilized blood cell graft[8, 9]
	Female donor for male recipient[8, 9]
	Parity of female donor[9]
	Cord blood graft (low risk)[8]
	HLA mismatch[9]
	Unrelated donor[8, 9]
	Older donor age[9]
Recipient factors	Older patient age[8, 9]
	Radiation (sclerotic GVHD)[10, 11]
	Busulfan (BOS)[12]