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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Introduction

***Consensus Key Point**: Moderate to severe cGVHD leads to excess morbidity and mortality and should be prevented.*

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

An ideal method to prevent chronic GVHD (cGVHD) after allogeneic hematopoietic cell transplantation (HCT) would remove the donor cells that cause cGVHD from the graft or allow them to attain operational tolerance of recipient alloantigens that cause cGVHD, such that systemic immunosuppression is no longer necessary. Recent studies have demonstrated substantial progress in preventing cGVHD, but this goal has not been fully realized because the underlying mechanisms that incite cGVHD are only partially known. Identification of the mechanisms that incite cGVHD in humans remains a challenge because at least 1 year of follow-up after HCT is needed to ascertain the development of cGVHD.

Mechanistic data strongly suggest distinct pivotal post-HCT cGVHD-inducing events involving certain donor T and B cell subsets and monocytes and recipient fibroblastic reticular cells. The evidence is derived from experiments using murine models and from studies of patient samples. Chronic GVHD may be prevented by removing certain activated donor lymphoid populations, albeit with immune consequences. The antigens that trigger cGVHD and GVT activity differ from those involved in pathogen defenses, but the extent of overlap and non-overlap between cGVHD and GVT-related antigens is not known. Pathogen-related antigens activate naïve cells that mature to effectors that clear the pathogen and establish a reservoir of memory cells for future defense. Pathogen-related antigens are typically cleared, but the recipient alloantigens that trigger cGVHD and GVT activity generally persist. The extent to which differences in functional responses between cGVHD and GVT activity and pathogen defense could be exploited to prevent cGVHD while preserving GVT activity and pathogen defenses is not known. This report reviews our current understanding of the etiologic factors, identifies knowledge gaps,

and suggests cellular targets and mechanisms relevant to benefits and risks in the design of clinical trials to prevent cGVHD.

Methods

Each working group was organized to encourage global engagement in the topic (see introduction to the series). 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, 2020 Consensus Conference. The manuscript was further revised for submission after additional suggestions from external reviewers, virtual Conference participants, and a 30-day public comment period.

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

Donor, recipient, and exogenous factors contribute to cGVHD genesis, and dynamic interactions between these factors and secondary insults lead to a final common pathway for developing cGVHD.[1] Studies in murine models[2, 3] and humans[4] indicate that events leading to cGVHD begin with tissue injury from the pretransplant conditioning regimen, which amplifies responses to alloantigens that trigger acute GVHD (aGVHD). Strategies that effectively reduce the risk of aGVHD, however, have not necessarily reduced the risk of cGVHD and vice versa, highlighting the need for further elucidation of the mechanisms that determine these outcomes. Approximately 30% of patients develop cGVHD with no prior overt aGVHD, either through a subacute graft-versus-host reaction or undefined independent mechanisms.[5] Better understanding of functional correlates and molecular drivers of immune cell subsets that mediate cGVHD is needed. Flow cytometric analyses alone cannot distinguish the cells that cause cGVHD from those that prevent graft rejection, mediate GVT activity and control infections.[6] Eliminating or blocking alloreactive T

cells before or early after HCT can prevent cGVHD, but this benefit can be offset by increased risks of graft rejection, recurrent or progressive malignancy, and opportunistic infections due to delayed immune reconstitution.

A working model of cGVHD development guides our approach to cGVHD prevention. Graft engineering prior to HCT and in vivo T cell depletion with ATG or cyclophosphamide remain areas of active investigation. Additional targets include B cells and monocytes. Data suggest that the risk of cGVHD is decreased by using younger donors, avoiding female donors for male patients, and by using cord blood grafts[7] and by using aspirated marrow cells instead of growth factor-mobilized blood cells.[8] Thus, donor selection serves as another point of intervention, which may become increasingly relevant as outcomes improve with alternative donor sources. **Figure 1** shows a working model of cGVHD inciting factors along with several potential points of clinical intervention that require further study.

Donor T cells subsets

Pre-transplant graft engineering remains an area of active investigation in cGVHD prevention since conventional/effector alloreactive T cells (Tcon/Teff) are critical for inciting and mediating cGVHD. Evidence suggests that subclinical pathogenic processes begin long before the distinct clinical manifestations of cGVHD become apparent. Our understanding of human cGVHD etiology is largely based on data derived from *in vivo* and *ex vivo* graft manipulation trials to prevent of acute GVHD (**Table 1,** perhaps better as a supplement). Clinical studies demonstrate that donor T cell dose is a major risk factor for the development of cGVHD. T cell depletion approaches have decreased the cGVHD incidence from 30% (bone marrow) and 50% (peripheral blood), to 10-40% for overall

Consensus Key Point: *Primary inciting cellular and molecular pathways leading to cGVHD arise from donor and host factors and are triggered early after HCT.*

Points of Intervention – Mitigation of Risk Factors for cGVHD Development

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

cGVHD and 10-20% for moderate and severe cGVHD.[9-14] These data are consistent with results showing that both allogeneic donor T cells and recipient alloantigens are necessary to develop cGVHD in murine models.[15]

Data in cGVHD and in other immune-mediated diseases have identified functionally distinct T cell subsets. Proinflammatory effector or conventional T (Teff/Tcon) cell subsets incite cGVHD, while other anti-inflammatory subsets such as regulatory T cells (Treg) attenuate disease. Initial approaches to induce apoptosis of rapidly dividing T cells early after HCT with methotrexate[16] did not ameliorate cGVHD. Greater success was achieved with approaches that deplete donor T cells via CD34 selection or alpha-beta T cell depletion or naïve T cells from the graft or use rabbit ATG in the conditioning regimen or high-dose cyclophosphamide after HCT to deplete donor T cells in vivo (**Table 1**).[9-13, 17-33] One unanswered question is why the incidence and severity of cGVHD are lower with umbilical cord blood (UCB) grafts than with marrow or mobilized blood cell grafts from older donors, even though UCB T cells are immunologically naïve. These results suggest that UCB contains fewer pathogenic Teff/Tcon or a greater proportion of Treg cells, a question that deserves further investigation.[13, 34-36] Th17 cells also appear to have a role in the etiology of cGVHD.[37]

Collectively, these data strongly suggest that the pathogenic T cells that initiate cGVHD are present at the time of stem cell infusion, even though clinical manifestations of cGVHD appear a year or more later. Removing these cells is currently an attractive approach, but as we further refine our understanding of the key T-cell subsets and their phenotypic profiles, more targeted approaches may be possible. The balance of Treg vs Teff/Tcon cells has a major role in the development of cGVHD. Treg/Teff ratios are low at the onset on cGVHD, and low ratios in patients without cGVHD predict a high future risk of cGVHD.[38, 39] Accordingly, adoptive transfer of ex-vivo-expanded Treg or *in-vivo* expansion mediated by low-dose IL-2 could decrease the risk of cGVHD.[40-44] Similarly, post-transplant cyclophosphamide (PTCy) depletes alloactivated Teff/Tcon with relative sparing of Treg. 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 cGVHD.[45-47] These approaches could reduce the risk of cGVHD while minimizing the risk of adverse effects.

Donor B cell subsets

The presence of allo- and autoantibodies in patients with cGVHD suggests a pathogenic role for B cells, and results from murine models have shown that antibody-producing B cells contribute to the disease[48, 49] and are necessary under in some models.[48, 50-52] How antibodies mediate cGVHD is an area of active investigation. One mechanism involves damage caused by antibody binding to thymic epithelial cell antigens.[50] The putative efficacy of B cell-depleting agents such as rituximab for treatment of cGVHD further suggests that pathogenic B cells contribute to clinical cGVHD.[53-55] Two prophylaxis studies have suggested that *in vivo* depletion of $CD20^+$ B cells at 2-3 months after HCT may decrease the risk of cGVHD.[56, 57] Global depletion of $CD20^+$ B cells can induce prolonged B lymphopenia in mice and in some patients, resulting in progression of cGVHD accompanied by delayed recovery of B cells.[46, 56, 58-62]

Consensus Key Point: *Agents that target cells that might not be critical in GVT reactions, like certain T cell subsets, distinct B cell subsets, monocytes or host lymphoid organ stromal cells represent prime candidates for further clinical studies.*

Points of intervention to mitigate cGVHD development

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

B cells that incite cGVHD are activated and primed for survival *in vivo*, suggesting a failure of regulation by B cell activating factor (BAFF). BAFF promotes survival and BCR-activation of B cells in mice that develop cGVHD (Jia W, Sarantopoulos, under revision at Blood Oct 6 2020). Potentially pathological B cells have a lowered B cell receptor (BCR) signaling threshold that enables hyper-reactivity.[63, 64] The BAFF tolerance checkpoint fails in cGVHD patients because levels of BAFF remain high enough to support aberrant B cells.[56, 58, 59] These results have refined our understanding of these potentially pathogenic B cells and suggest that CD20 may not be the optimal target.[61] Thus, selective depletion of constitutively activated, alloreactive B cells could be

a more effective strategy to reduce the incidence and severity of cGVHD. Not only could this approach attenuate cGVHD, but it would allow immune recovery of a comprehensive, diverse, peripheral B-cell compartment under physiologic homeostatic control.[64-66] Alternatively, low numbers of B regulatory cells (Breg) have been associated with aberrant immune recovery and cGVHD,[45, 46] suggesting that strategies to expand these cells *in vivo* could decrease the risk of cGVHD

Donor monocytes/macrophages

The divers of T cell dysregulation in cGVHD include macrophages, although their role in humans has been difficult to investigate. Results from murine models of pulmonary cGVHD have supported a significant role for donor-derived alternatively activated macrophages (M2) that drive Th2- and Th17-cell activation.[67]

Recipient fibroblastic reticular cells

Certain recipient stromal cells with immune functions in secondary lymphoid organs (SLO) have a role in the etiology of cGVHD. In mice, lymph node damage impairs T and B cell interactions through loss of fibroblastic reticular cells (FRC) that are necessary to induce tolerance.[3, 68] FRCs, and potentially other recipient stromal cells, can also incite cGVHD via Notch ligand interactions that lead to aberrant activation of lymphocytes.[49, 69, 70] In patients with cGVHD, SLO damage is suggested by the high numbers of circulating follicular T and B cells, and post-GC plasmablast-like cells that typically reside in SLO.[71] These CD4 T follicular helper (Tfh) cells interact with B cells, leading to increased plasma cell-like activation in active clinical cGVHD.[72] Whether the risk of cGVHD could be averted or diminished by optimizing the function of these 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 operational tolerance supports this possibility.[45, 46, 73]

Studies of murine models have shown that interactions between FRCs and B cells in recipient lymphoid organs may cause cGVHD.[49] FRCs are increased in number early after transplant and they have increased BAFF transcription (Jia, W under revision at Blood). FRCs promote cGVHD because they are defective in their capacity to present tissue antigens.[3] Other myofibroblasts are pathologically activated in cGVHD[74] and may incite pathways leading to cGVHD. The clear association of cGVHD with the level of recovery of certain immune cell subsets[58] strongly 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.

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

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 cGVHD through antigen exposure and presentation, with damage propagated by infection, microbiome disruption, and loss of oral and gastrointestinal mucosal integrity.[75] Tissue signals drive recipient alloantigen presentation in murine models, inciting the aberrant activation cascade that leads to cGVHD.[2] Approaches to minimize tissue injury in the preparative regimen with agents such as c-kit antibody could mitigate this contribution to cGVHD.[76]

Recipient thymic epithelial cell dysfunction

Impaired thymopoiesis is highly associated with cGVHD.[77] The thymus incurs damage from the preparative regimen, immunosuppressive medications and donor T cells.[50] 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 cGVHD.[15] AIRE dysfunction and loss of intrathymic group 3 innate lymphoid cells contribute to failure of

negative selection.[78, 79] The extent to which this mechanism applies in human HCT is not known. In contrast, studies of human blood samples have shown that T cell reconstitution after HCT is derived primarily from expansion of mature T cells in the graft that have a restricted TCR repertoire, with far less contribution from marrow-derived cells that differentiate in the thymus. These T cells have a restricted TCR repertoire and little evidence of thymic derivation as indicated by the presence of T receptor excision circles and small thymic size by radiographic imaging.[39, 80-84] These results suggest that lack of thymic recovery and failure to generate a diverse thymic-derived naïve T cell repertoire could contribute to lymphoid dysregulation in the etiology of cGVHD. The lower cGVHD rates in children compared to adults raise the question of whether thymic recovery could have a protective effect by maintaining effective negative selection and robust production of Treg cells.[77, 85-90]

Experiments with murine models have supported a role for androgen withdrawal, IGF-1 and keratinocyte growth factor (KGF) in thymic recovery.[90-93] These results have motivated trials to test whether androgen suppression, IGF-1 supplementation and keratinocyte growth factor can decrease the risk of cGVHD. Results with KGF have not been encouraging,[94] and results with other agents have not been reported.

Key Consensus Point: *Secondary insults may occur at any time after HCT.*

Develop Risk Mitigation Strategies based on second insults leading to cGVHD

- HLA matching and other genetic risk factors
- Infection prevention
- Tissue damage prevention

Non-immune organ tissue Damage and cGVHD Development

Certain exogenous events can be considered second insults that incite cGVHD by damaging recipient tissues post-HCT, and may be necessary steps for certain forms of cGVHD. 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.[95] Tissue damage related to viral and other infections may incite cGVHD. CMV and HSV reactivation

have been linked to cGVHD[96, 97] and anti-viral prophylaxis may offer some protection against cGVHD. Pre-transplant decreased surfactant, likely due to injured epithelia, confers an increased risk of lung GvHD.[98, 99] Elevated collagen type V, a marker of alveolar epithelial injury, has also been linked to active pulmonary cGVHD.[100-102] 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 cGVHD are limited. Periodontal disease leads to both gingival inflammation and breakdown. In one study, oral microbiome changes were associated with oral cGVHD.[103-105] Likewise, loss of gastrointestinal microbial diversity has been linked to GVHD.[106-108] These data support studies that address the interaction between specific tissues and the local microbiome on cGVHD development.[106]

Factors that incite sclerotic skin and connective tissue manifestations of cGVHD remain less well known. The association of sun exposure and local mechanical stress with focal cutaneous manifestations of cGVHD 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 cGVHD.

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

Balancing the risks of moderate or severe cGVHD versus the risks of graft rejection, delayed immune reconstitution and recurrent or progressive malignancy poses a key issue in designing trials to prevent cGVHD. 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

***Consensus Key Point**: For patients with hematological malignancies, a risk assessment model for moderate-severe cGVHD 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 cGVHD development

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

decrease the magnitude of tissue damage that might trigger cGVHD, it could also increase the risk of relapse. Interventions that decrease the numbers, activation or survival of donor T cells could decrease the risk of cGVHD but could also decrease their ability to prevent graft rejection and recurrent or progressive malignancy. Highly intensive immunosuppressive regimens could decrease the risk of cGVHD but could also delay immune recovery, increase susceptibility to infections, and possibly increase the risk of recurrent or progressive malignancy. Although interventions to prevent cGVHD could increase the risks of graft rejection, delayed immune reconstitution and opportunistic infections, effects on the risk of recurrent or progressive malignancy 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 cGVHD? Several large studies have shown potent GVT effects associated with the presence of cGVHD by NIH criteria and an increased risk of recurrent or progressive malignancy in patients who did not develop cGVHD.[109-111] Mild cGVHD has been associated with improved overall survival, while moderate or severe cGVHD has been associated with an increased risk of non-relapse mortality.[112, 113] During the first 18 months after HCT, patients without cGVHD who continued immunosuppressive medications had the highest risk of relapse.[110] Beyond 18 months after HCT, patients who did not experience cGVHD showed the highest risk of relapse even thought 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 cGVHD.[110]

Some of the therapeutic interventions with the greatest effect in preventing cGVHD have been associated with high relapse rates (**Table 1**). In addition, many studies have shown that the magnitude of GVT effects differ according to disease type and disease status.[111, 114] Some evidence suggests that myeloid malignancies are more immunogenic and responsive to GVT activity, but further studies are needed to determine the extent of differences in susceptibility to

GVT activity between different diseases. When evaluating the potential success of an intervention to prevent cGVHD, it is imperative to consider risk stratification for recurrent or progressive malignancy in the eligibility criteria. The presence or absence of measurable residual disease (MRD) in the marrow of patients with acute leukemia at the time of HCT has a major effect on the risk of relapse after HCT and should be taken into account in designing clinical trials to prevent cGVHD. One approach of great interest would be to test agents that could simultaneously target malignant cells and cGVHD.[115]

Emerging data suggest that adoptive transfer of certain NK subsets [116] or invariant NKT[117] or gamma delta T cells[118] could minimize the risk of cGVHD while preserving GVT benefits. Future studies should focus on testing whether these lymphocyte populations could be used to prevent cGVHD without impairing GVT activity.

V. Critical questions and answers about cGVHD prevention trials

> ***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 cGVHD risk stratification*
>
> Points of intervention to mitigate cGVHD development
> - Use translational data to identify populations at high risk of cGVHD
> - Use translational data to identify novel targets

The selection of interventions and approaches to test for prevention of cGVHD should be based on an understanding of the underlying mechanisms that initiate the processes leading to development of cGVHD, 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 cGVHD.

1) What preventive agents and approaches are most promising?

The key considerations include the strength of the efficacy data for the cGVHD intervention or approach versus the influence on other HCT outcomes, including prevention of graft rejection, viral infections and recurrent or progressive malignancy (i.e., relapse).

Approaches that prevent cGVHD 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. To understand the influence of an investigational product or approach on relapse, it will be crucial to document the underlying risk of relapse as accurately as possible. In patients with acute leukemia, the presence of MRD at the time of HCT reflects not only residual disease burden but also disease sensitivity to prior therapies, and thus can be the single strongest predictor of relapse after HCT.[119, 120] Most reports of cGVHD 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 disease risk index (DRI) will be important to understand the impact of specific approaches on the risk of relapse.[121] It may be possible to refine graft engineering approaches as we begin to understand the mechanisms leading to cGVHD. 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,[122] adjusting the numbers of T cells in the graft, and the timing of *in-vivo* cell depletion or expansion strategies and adoptive transfer strategies, or ways to improve outcomes with cord blood grafts. In addition, altering other aspects of the trial, for example, by increasing tumor control through another avenue, may also enhance outcomes while minimizing the risk of cGVHD.

2) Who should be enrolled in cGVHD prevention trials?

Trial inclusion and exclusion criteria should enrich for patients at high risk of moderate-severe cGVHD 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 cGVHD. 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**). Eligibility criteria

should be designed to include patients at high risk of cGVHD and to exclude patients at high risk of complications that could be caused by the study intervention. Selecting patients with non-malignant diseases would obviate the risk of relapse, but most patients have malignant diseases. Patients with malignant diseases should be included in prevention studies after careful consideration of the relapse risk. Children should be considered separately in assessing the risk/benefit ratio, because the risk of cGVHD is much lower than in adults. Future eligibility criteria could include biomarkers that have a high positive predictive value for development of cGVHD. If the risk of a study intervention is low, enrollment of patients with a low risk of developing cGVHD may be justified. If the risk of the study intervention is high, however, enrollment of patients with a low risk of developing cGVHD 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 cGVHD prevention trials?

3.1) Primary efficacy endpoint

In pivotal cGVHD prevention trials intended for regulatory review, survival without moderate or severe NIH-defined cGVHD (cGVHD-free survival) should serve as the primary endpoint. Results for this endpoint have been reported in only one study.[12] In this randomized prospective study that compared standard of care prophylaxis with or without added ATG, the rate of moderate or severe cGVHD-free survival at 2 years was 44% (95% CI, 34% to 52%) in patients who received standard post-transplant immunosuppression with tacrolimus and methotrexate and 48% (95% CI, 38% to 58%) in the ATG group.[12] Cumulative incidence estimates of moderate or severe cGVHD are not appropriate as the primary endpoint, because they can be decreased by a high incidence of death as a competing risks (i.e., death from relapse). Chronic GVHD requiring systemic treatment could serve as a functional endpoint definition instead of moderate to severe cGVHD, 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 of cGVHD develops within one year, but longer follow-up would be highly desirable for some secondary endpoints.

In earlier phase studies, an endpoint that captures an effect linked to cGVHD 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 cGVHD, an early phase trial could be designed around these immunological endpoints, provided that the linkage to prevention of cGVHD is strong.

3.2) Composite endpoints

Composite endpoints such as cGVHD-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 cGVHD (i.e. relapse, death, and severe acute GVHD) can confound the interpretation of the most relevant failure event (i.e., cGVHD). GRFS is appropriate if it is anticipated that the study intervention is likely to decrease the incidence of both acute and chronic GVHD. For cGVHD prevention studies, CRFS is preferred. In all studies, separate reporting of each component in composite endpoints and tabulation of the causes of death are needed to understand the benefits and risks of the study intervention.

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 or severe cGVHD. Therefore, randomization may be preferred for phase 2 studies, if feasible. Participants in trials to prevent cGVHD 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 cGVHD 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 or severe cGVHD 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 or severe cGVHD, thereby informing the design and interpretation of results in future trials.

SUMMARY

Below we summarize recommendations for studies over the next 5 years

1. Elucidate the inciting cellular and molecular pathway determinants of immune operational tolerance after HCT.

1.a*. Use primary patient samples and murine models to determine how recipient and donor characteristics incite or attenuate the development of cGVHD.*

1.b*. Define roles for recipient tissues and organs in the initiation of cGVHD.*

1.c*. Determine how second insults after HCT incite moderate-severe cGVHD.*

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

3. Work toward defining a dynamic individualized risk stratification strategy that predicts risks of relapse, graft failure, and viral infections, and balances these against the risk of developing moderate-severe cGVHD to identify patients who would most benefit from cGVHD prevention trials.

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

Table 1. Clinical studies that have informed us about potential early points of intervention and about the etiology of cGVHD.

Approach	In vivo Ex-vivo	Study design	Cells targeted	Backbone GVHD ppx	Cohort	%MRD+	Donor	Stem cell source (n)	%Moderate to severe* cGVHD	%Any cGVHD	%Relapse	%Graft failure	%OS	Ref.
Anti-CD20 Alpha/ß	Ex-vivo	Ph1/2	Alpha/ß T, B	ATG pre	Ped./ HM/TBI	Unk	Haplo	PB (81)	0	0	24	2	71	[17]
Anti-CD20 Alpha/ß	Ex-vivo	Pilot	Alpha/ß T, B	ATG pre, CNI ± MTX	Ped./ HM/TBI	18	Haplo/MUD	PB (33)	12	30	30	0	67	[18]
Anti-CD20 Alpha/ß	Ex-vivo	Retro	Alpha/ß T, B	ATG pre	Ped/ NM	N/A	Haplo	PB (14)	21	Unk	N/A	14	84	[19]
Anti-CD20 Alpha/ß	Ex-vivo	Ph1/2	Alpha/ß T, B	ATG pre + rituximab	Ped/ NM	N/A	Haplo	PB (23)	0	0	N/A	17	91	[20]
ATG vs. None	In vivo	Ph3	ATG rabbit	CNI+MTX	Adult/ HM	Unk	MUD/ MRD	PB (155)	6 vs. 33	27 vs. 64	32 vs. 26	0 vs, 1	74 vs. 78	[10]
ATG vs. None	In vivo	Ph3	ATG rabbit	CNI+MTX	Adult/ HM	Unk	MUD	PB (164) BM (37)	12 vs. 45	30 vs. 60	33 vs 28	Unk.	55 vs. 43	[9, 21]
ATG vs. None	In vivo	Ph3	ATG rabbit	CNI+MTX	Adult/ HM	Unk	MUD	PB (196) BM (49)	12 vs. 33	16 vs.38	32 vs. 21	21 vs. 6	59 vs. 74	[12]
ATG vs. None	In vivo	Ph3	ATG rabbit	CNI+MTX or MMF	Adult/ HM	Unk	MUD/MMUD	PB (173) BM (23)	13 vs. 29	22 vs. 33	11 vs 16	3 vs. 2	74 vs 79 @6 mo	[11]
ATG vs. None	In vivo	Ph3	ATG rabbit	CSP+MTX+ MMF	Adult/ HM	Unk	MRD	BM+PB (101) PB (153) BM (9)	8.5 vs. 23	28 vs. 53	21 vs. 15	0 vs. 0	69 vs. 70	[22]
AntiCD45RA + CD34 selec.	Ex vivo	Ph2	Naive T/ CD34-	Tacrolimus	Adult/ HM/TBI	37	MRD	PB (35)	3	9	21	0	78	[13]
CD34 selec.	Ex vivo	Ph2	CD34-	None	Adult/ HM/TBI	Unk	MRD	PB (44)	7	18	24	0	60	[23]
CD34 selec	Ex vivo	Retro	CD34-	ATG	Adult/ HM/TBI	Unk	MRD>7/8	PB (241)	1	5	22	<1	57	[24]
PTCy	In vivo	Ph2	Activated T	CSP	Adult/ HM	49	MRD/MUD	PB (45)	30	Unk	17	2	70	[25]
PTCy	In vivo	Retro	Activated T	CNI+ MMF	Adult/ HM	58	Haplo	BM (104)	Unk	30	44	10	45	[26]
PTCy	In vivo	Retro	Activated T	None	Adult/ HM	58	MRD/MUD	BM (297)	Unk	12	37	5*	72	[27]
PTCy	In vivo	Ph2	Activated T	None	Adult/ HM	47	MRD/MUD	BM (92)	14	14	22	5	67	[28]
PTCy	In vivo	Retro	Activated T	CNI+MMF	Adult/ HM	34	MUD	BM (150)	15	45	24	8	57	[29]
PTCy	In vivo	Retro	Activated T	None vs. CNI + MTX	Ped/ HM/TBI	45 vs. 22	MRD	BM (29)	0 vs 0	0 vs 6	45 vs. 44	0 vs 0	54 vs. 58	[30]
PTCy	In vivo	Retro	Activated T	None	Ped/NM	N/A	Haplo	BM (27)	0	24	N/A	22	78	[31]
PTCy	In vivo	Ph2	Activated T	CNI + MMF	Both/HM	Unk.	Haplo	BM (68)	13	Unk	51	13	36	[32]
PTCy	In vivo	Ph1/2	Activated T	None	Adult/HM		MRD/MUD	BM (117)	3	10	44	3	55	[33]
CB vs. MUD		Retro	(memory) vs. none	CNI + MTX or MMF	Both/HM	31-39	Cord >3/6 MUD	PB (237) BM (107) CB (140)	Unk but no. diff.	Unk but no. diff.	15 vs. 24	Unk	71 vs. 63	[36]
CB vs. MUD		Retro	(memory) vs. none	CNI + MTX or MMF	Adult/HM/ TBI	Unk	Cord >3/6 MUD	PB (361) BM (185) CB (116)	23 vs. 34	39 vs. 42	22 vs. 25	8 vs. 3	44 vs. 43	[123]

CB vs. MUD /MSD		Retro	(memory) vs. none	CNI + MTX or MMF	Both/HM/ TBI	Equiv.	Cord>3/6 MUD/MSD	PB (275) BM (81) CB (128)	Unk	26 vs. 43-47	15 vs. 37-43	10 vs 0	Unk, LFS: 51 vs.33-48	[34]
CB		Ph2	(memory)	CNI + MMF	Adult/NM	N/A	Cord >3/6	CB (26)	12	36	N/A	12	85	[124]
Anti-CD20	In-vivo	Ph2	B cells	Unk	Adult/HM/ RIC60%	46 vs. 41	MRD MUD	PB (65)	31 vs. 49	48 vs. 60	34 vs. 28	N/A	71 vs. 56	[56]
CB Treg vs. CB control		Ph1	Activated T	Siro + MMF	Adult/HM	Unk	mmMUD Cord >3/6	CB (11)	Unk.	0 vs 14	33 vs. 40	9 vs. 14	81 vs. 61 @ 1 yr	[125]
PB vs. BM		Ph3		CNI + MTX	Adult/HM		MUD/ mmMUD	PB (273) BM (278)	48 vs. 32	53 vs. 41	30 vs. 30	2 vs. 6	46 vs. 51	[8]

*Some studies report extensive cGVHD. Footnotes to be added.

Table 2. Risk factors for development of chronic GVHD

Donor factors	HLA mismatch[126]
	Unrelated donor (except CB)[7, 126]
	Older donor age[126]
	Female donor for male recipient[7, 126]
	Parity of female donor[126]
	Mobilized blood cell graft[7, 126]
	Cord blood graft (low risk)[7]
	Genetic polymorphisms[127-129]
Recipient factors	Older patient age[7, 126]
	Genetic polymorphisms[127-130]
	Radiation for sclerotic GVHD[131, 132]
	Busulfan for BOS[133]

Figure Legends

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 cGVHD, 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 deplete alloantigen-activated T cells 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 aGVHD, and the yellow triangle denotes the onset of cGVHD, 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 cGVHD 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 cGVHD.

Figure 3. Critical considerations in the design of cGVHD prevention trials. Under the assumption that the anticipated efficacy of the study intervention is high enough to warrant the effort and cost of conducting a trial, the eligibility criteria should be defined to selects patients at high risk of cGVHD (shown on the X-axis) so that potential benefits outweigh the intervention risks, especially among patients not destined to develop cGVHD. Potential benefit is proportional to the positive predictive value but is not a direct measure of potential benefit. Risks should be assessed in terms of frequency and severity. High-risk interventions are acceptable only for patients at high risk of cGVHD, while lower-risk interventions may be acceptable for patients at lower risk of cGVHD.

Figure 1

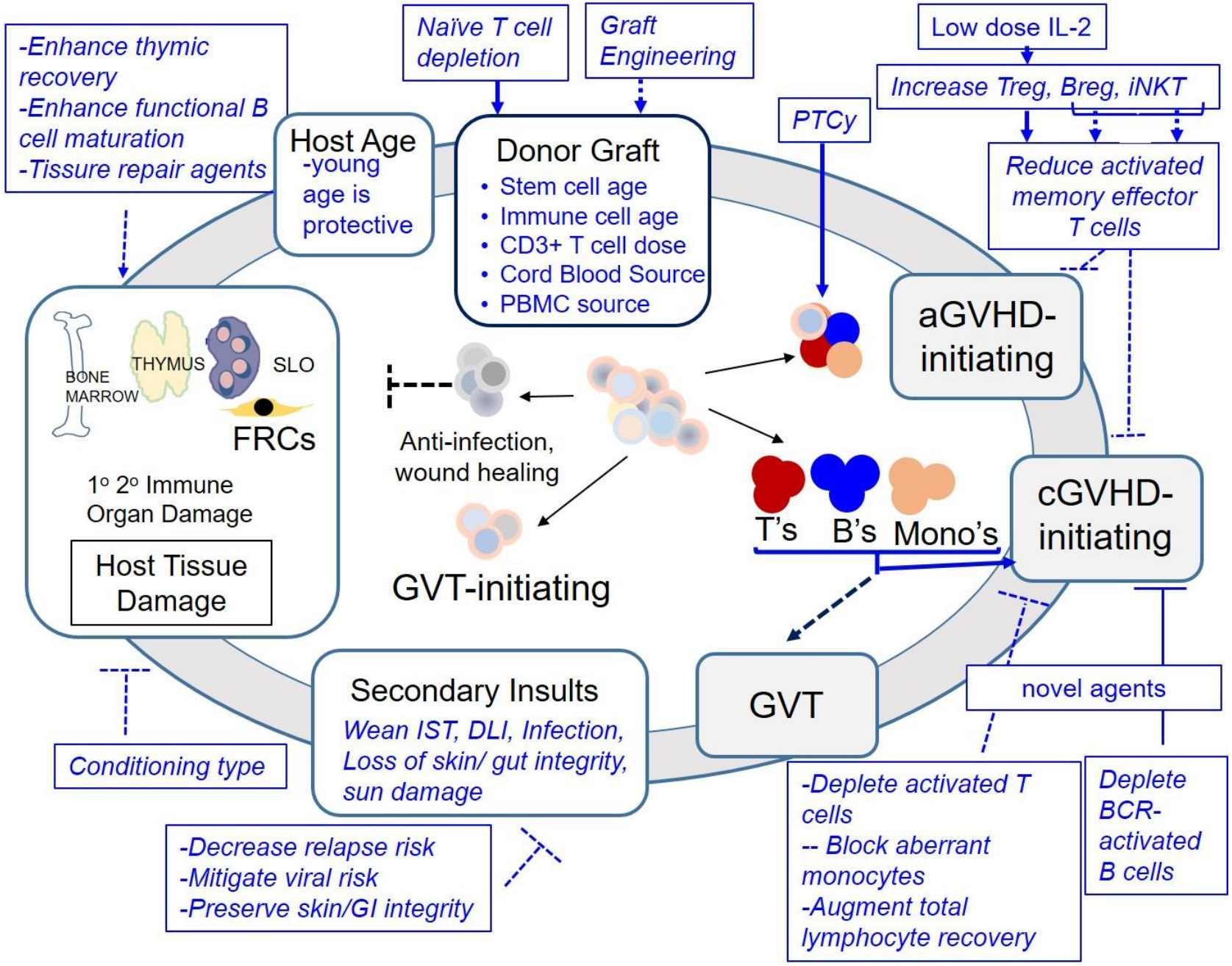

-Enhance thymic recovery
-Enhance functional B cell maturation
-Tissure repair agents
Naïve T cell depletion
Graft Engineering
Low dose IL-2
Increase Treg, Breg, iNKT
PTCy
Reduce activated memory effector T cells
Host Age
-young age is protective
Donor Graft
• Stem cell age
• Immune cell age
• CD3+ T cell dose
• Cord Blood Source
• PBMC source
aGVHD-initiating
BONE MARROW
THYMUS
SLO
FRCs
1º 2º Immune Organ Damage
Host Tissue Damage
Anti-infection, wound healing
GVT-initiating
T's
B's
Mono's
cGVHD-initiating
Conditioning type
Secondary Insults
Wean IST, DLI, Infection, Loss of skin/ gut integrity, sun damage
GVT
novel agents
-Decrease relapse risk
-Mitigate viral risk
-Preserve skin/GI integrity
-Deplete activated T cells
-- Block aberrant monocytes
-Augment total lymphocyte recovery
Deplete BCR-activated B cells

Figure 2

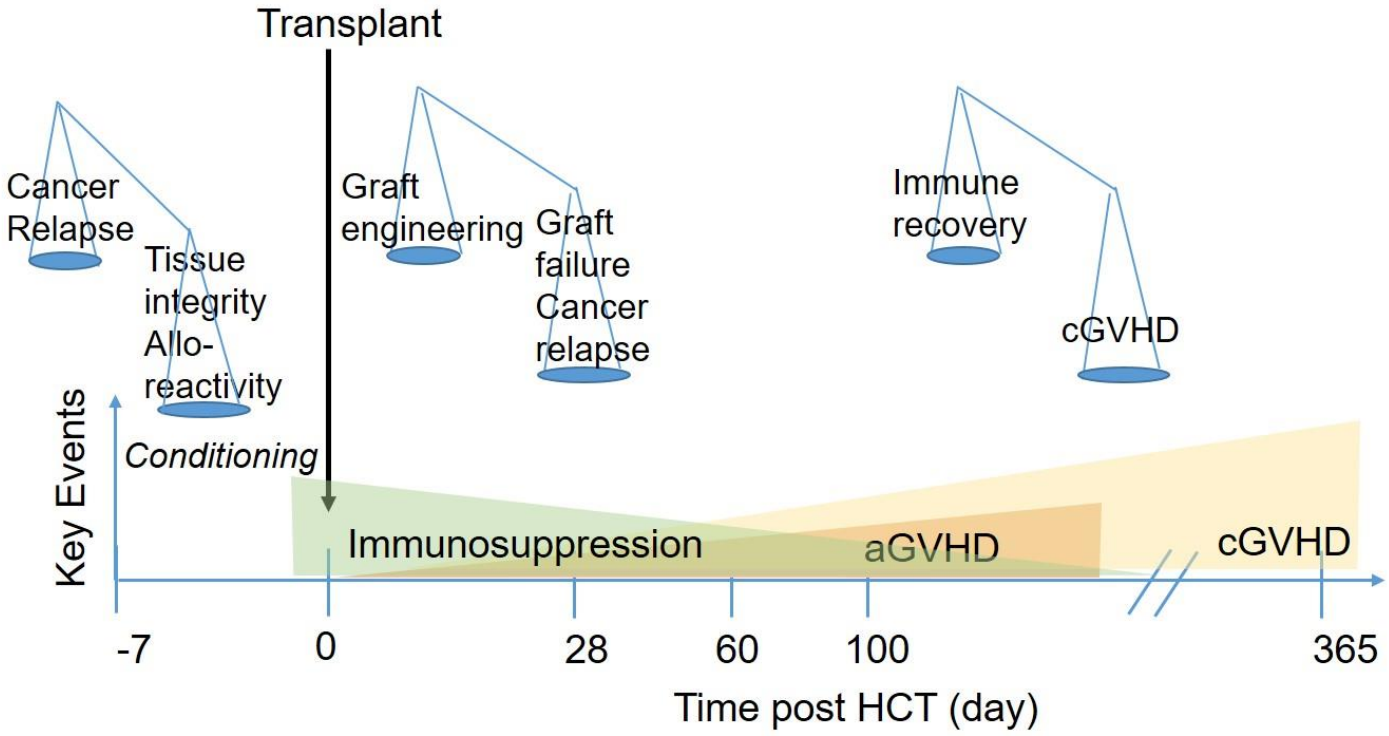
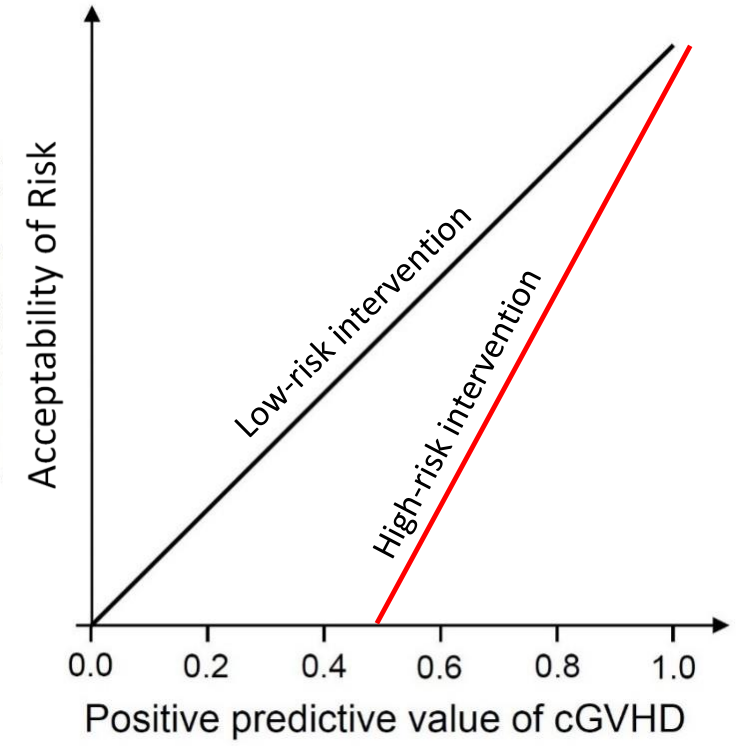

Transplant
Cancer Relapse
Tissue integrity Allo-reactivity
Graft engineering
Graft failure Cancer relapse
Immune recovery
cGVHD
Key Events
Conditioning
Immunosuppression
aGVHD
cGVHD
-7
0
28
60
100
365
Time post HCT (day)

Figure 3


Acceptability of Risk
Low-risk intervention
High-risk intervention
0.0
0.2
0.4
0.6
0.8
1.0
Positive predictive value of cGVHD

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