Systematic Review Protocols:
Interventions Used to Manage Patients Undergoing Hematopoietic Stem Cell Transplantantion

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1. Background for the Planned Systematic Reviews

Allogeneic hematopoietic cell transplantation (HSCT) represents a multi-faceted and complex health care intervention that can be lifesaving for patients with hematologic cancers, other serious blood or immune disorders, and inherited metabolic conditions.[1,2] Optimization of strategies to improve patient outcomes has evolved gradually as transplant activity has increased globally over the past 25 years. With increasing activity, it is now possible to address questions regarding drug effectiveness and safety in HSCT using available evidence from randomized controlled trials. To date, however, clear recommendations regarding treatment selection within an array of transplant-related interventions remains lacking. In particular, it remains challenging to quantify the strength of evidence that underpins many treatment and prevention strategies used currently by transplant centres. This is due to a lack of direct or indirect comparisons for particular interventions, and has led to wide variation in institutional protocols for many aspects of care in HSCT.[3-6] Ready access to the foundation of evidence supporting practices in allogeneic transplantation remains limited. Many of the drug interventions in HSCT are costly and are associated with significant potential toxicities, such as prolonged immune suppression, severe mucositis, and risk of renal and liver injury, making a formal analysis of safety and effectiveness analysis highly relevant for the improvement of care and the sustainability of transplantation programs.

Several major aspects of care in HSCT require delicate balancing of safety and effectiveness to ensure the best outcomes for patients. Three particular topics have been identified for study in the proposed work:

1. When undergoing HSCT, patients must initially have their underlying disease eradicated.[7] This is accomplished clinically by administration of a conditioning regimen immediately prior to the transplant. Conditioning regimens (a mixture of chemotherapy and/or irradiation) have evolved over time to be prescribed in different strengths: *myeloblative* regimens, *non-myeloablative* regimens, and *reduced intensity* regimens.[7,8] The best choice of conditioning therapy remains unclear, and a robust systematic review is needed to address this knowledge gap.

2. Graft versus host disease (GVHD) is a challenging complication that frequently occurs following allogeneic HSCT. GVHD involves the transplant graft identifying the recipient as a foreign agent, and subsequently results in the transplanted immune cells attacking the body of the recipient.[9] A variety of approaches to management involving pharmacologics such as steroids, calcineurin inhibitors, mammalian target of rapamycin (mTOR) inhibitors and other agents are possible. Considerable inter-institutional variation in clinical practice is a barrier to improving the success of prevention and treatment strategies and improving patient safety. Uniformity in clinical practice in the prevention and treatment of GVHD based on robust syntheses of evidence will allow improvements in patient outcomes.

3. While HSCT has greatly improved outcomes in patients with malignant and non-malignant conditions, infections (bacterial, fungal, and viral) still account for considerable mortality in this patient population, and treatment is associated with challenges and high costs.[10,11] A variety of pharmacologic agents may be used in clinical practice. The optimal choices of agents for infection prevention and treatment continue to evolve with the advent of newer and increasingly expensive antibiotics, antivirals, and antifungals. There is a need to quantify the benefits and harms of the different interventions using a systematic approach to resolve the best treatment choices.

1.1 Context for this Work

Knowing the optimal treatment and prevention strategies that balance effectiveness with safety will allow transplant centres to improve patient outcomes and use resources most responsibly. Provincial stem cell

committees typically report to provincial cancer care organizations and/or directly to the Ministry of Health (e.g., the Ontario Stem Cell Transplant Committee reports to Cancer Care Ontario and to the Ontario Ministry of Health and Long-term Care). Physician members of these transplant committees are united in a national professional organization, the Canadian Blood and Marrow Transplant Group (CBMTG), that can initiate leading practice guidelines to assist in provincial funding discussions. The pan-Canadian Oncology Drug Review will work in conjunction with members of the CBMTG to lobby provincial reimbursement committees. Having a national body to provide a united approach will be a key to successfully translating the results of our work.

The chief goals of our efforts are to: (1) provide an annotated foundation of best evidence that can allow us to identify areas of transplant care that are understudied; (2) assess the quality of evidence that underpins institutional practice in HSCT; (3) provide evidence to key knowledge users to enable development of recommendations for transplant centres regarding the effectiveness and safety of specific interventions; and (4) share evidence with trialists in this field seeking to plan future clinical trials that are most needed and which will have the greatest impact on improved patient outcomes. Systematic reviews of evidence from RCTs can facilitate network meta-analyses of treatment and prevention strategies to compare outcomes and identify optimal practices.

1.2 Current Gaps in the Literature

While some systematic reviews and guidance have been published on specific interventions for HSCT, there have been no comprehensive efforts to establish guidelines based on networks of evidence and formal network meta-analyses of randomized trials. In particular, the literature is currently lacking analyses of indirect comparisons that include important effectiveness and safety outcomes. These comparisons can be achieved through network meta-analysis and would be transformative for transplant physicians, their patients, and funding bodies. A search of the Agency for Healthcare Research and Quality database identified only 9 HSCT guidelines, including 4 guidelines for treatment and/or prevention of GVHD, 2 on the treatment and/or prevention of infections, and 1 on conditioning regimens. The single network meta-analysis located in a preliminary search focused on prevention of GVHD only[12], reported only on the incidence of GVHD, and did not report or assess other key clinical outcomes crucial to the evaluation of GVHD interventions, such as survival, disease relapse, and infectious complications. The authors also appeared to include data twice or more for patients included in published studies that had follow-up results published later, thereby potentially overestimating the effects of studies with longer follow-up. GVHD is further complicated by different effects on different organs and this was not considered in the comparisons. To our knowledge, no other networks have been created, and a comprehensive scoping review of drug effectiveness or safety has not been performed for HSCT conditioning regimens or anti-infective strategies.

Research Questions to be Addressed in This Work

We plan to conduct a series of three systematic reviews addressing the following research questions:

1. *What are the relative safety and effectiveness of competing conditioning regimens (and groupings of conditioning regimens) used for allogeneic HSCT?*
2. *What are the relative safety and effectiveness of different pharmacologic strategies to prevent and treat graft versus host disease following allogeneic HSCT?*
3. *What are the relative safety and effectiveness of different drugs used to prevent and treat bacterial, viral, and fungal infections following allogeneic HSCT?*

This document outlines three protocols for the planned systematic reviews. They have been structured to follow Cochrane guidance for protocols of systematic reviews incorporating network meta-analyses.[13] Each review is anticipated to incorporate network meta-analyses for comparison of multiple interventions of clinical relevance to clinicians treating patients und ergoing allogeneic HSCT. Where methods are common and comparable across research questions, we have described steps in detail for research question 1 and referred back to them for research questions 2 and 3.

RESEARCH QUESTION 1:

Comparison of Conditioning Regimens for Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplant

2. Research Question 1: What are the relative safety and effectiveness of competing conditioning regimens following allogeneic blood stem cell transplantation?

2.1 Description of the Condition

In the 1950s, animal models demonstrated that after low-dose total body irradiation, death could be averted by the transplantation of bone marrow from an identical twin (syngeneic) or from the animal's own (autologous) stored bone marrow.[14] With the discovery of human leukocyte antigens (HLAs) in the 1960s and their influence on graft survival, bone marrow transplants from genetically different individuals (allogeneic) that had matched HLA profiles became feasible, and allogeneic hematopoietic stem cell transfer (HSCT) became a viable potential treatment option for hematologic disease.[1] During more recent years, HSCT has been used to treat a variety of cancers and other diseases, including acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, juvenile chronic myeloid leukemia, chronic lymphocytic leukemia, myelodysplastic syndromes, myeloproliferative disorders and sickle cell anemia, amongst other conditions.[1] Worldwide, the vast majority of HSCTs are used to treat lymphoid and hematologic cancers, with nearly half performed to treat acute leukemias.

In brief, allogeneic HSCT involves either complete or partial ablation of the hematopoietic system and profound immunoablation to facilitate engraftment of the donor blood and immune system, achieved through transplantation of healthy donor hematopoietic stem cells. These stem cells (1) produce daughter stem cells, so as to be self-renewing; and (2) differentiate to become the primitive progenitors of the entire lymphohematopoietic system that will give rise to specialized precursors of the various cell lineages.

Immediately prior to transplantation, patients have residual underlying disease and their cellular immunity eradicated through the administration of a *conditioning regimen*. Conditioning regimens can consist of a mixture of chemotherapy and/or total body irradiation and other agents, and can be prescribed in different strengths: *myeloblative*, *non-myeloablative*, and *reduced-intensity* regimens.[7,8] While the intent of myeloablative regimens is to completely eradicate the patient's disease, the non-myeloablative regimens eradicate only a portion of cancerous cells, and instead rely on the *graft-versus-tumor effect*—in which donor-derived alloreactive immune cells eliminate cancer cells over time—providing immune surveillance that can eliminate the remaining diseased cells. Because myeloablative regimens are associated with greater transplant-related mortality with increasing patient age, non-myeloablative regimens evolved to enable older and more medically fragile patients to undergo HSCT.[7,8] Reduced-intensity regimens can be considered an intermediate category. Currently, the best choice of conditioning therapy to treat patients before transplantation remains unclear, and a robust systematic review is needed to address this knowledge gap.

2.2 Description of the Interventions and How They Work

A variety of interventions are used in conditioning regimens administered to patients undergoing HSCT. Generally speaking, when used together these agents work to destroy both healthy and diseased bone marrow, with the goals of eliminating disease and preventing graft rejection. Conditioning regimens primarily are formed of various pairings of the following components:

- ***Antithymocyte globulin (ATG)***: Antithymocyte globulin dramatically reduces the number of circulating T-lymphocytes through cell lysis. This reduces host immune response in the immediate post-transplant phase, but also increases the risk of infection.
- ***Busulfan (BU)***: Busulfan is an alkylating agent that selectively targets rapidly dividing cells of the bone marrow, including stem cells, acting through formation of intra-strand DNA crosslinks that prevent DNA replication and cause cell death.[15] In high doses, it is myeloablative and can be used in place of total body irradiation. With other drugs, it is commonly used in conditioning regimens for patients with acute or chronic leukemias. Significant adverse effects can occur with

its use due to highly v ariable pharmacokinetics which have been partly reduced through greater use of intravenous rather than oral formulations. The most commonly used non-radiation-containing conditioning regimen is cyclophosphamide with busulfan.

- ***Cyclophosphamide (CY)***: Similar to busulfan, cyclophosphamide is an alkylating agent that causes intra-strand as well as inter-strand DNA crosslinks, preventing DNA replication and causing cell death. It preferentially targets lymphoid cells and is highly immune suppressive. Associated important toxicities include hemorrhagic cystitis and secondary cancers.
- ***Etoposide***: Etoposide is a topoisomerase inhibitor that acts by rapidly dividing cancer cells to break DNA strands, inhibiting DNA synthesis and promoting programmed cell death.[16] It can affect all cell lines in the bone marrow, leading to immunosuppression, anemia, and thrombocytopenia, with their associated side effects. It may be combined in high doses with total body irradiation as a conditioning regimen.
- ***Fludarabine***: Fludarabine is a purine analog that acts on ribonucleotide reductase and DNA polymerase to inhibit DNA synthesis, targeting both dividing and non-dividing cells.[17] It has relatively high specificity for both healthy and diseased blood cells, and causes suppression of all cell lines, resulting in immunosuppression, anemia, and thrombocytopenia, with their associated side effects. Fludarabine is often used in reduced-intensity and non-myeloablative conditioning regimens.
- ***Melphalan***: Melphalan is an alkylating agent that causes inter-strand crosslinks in DNA, inhibiting DNA and RNA synthesis and causing programmed cell death.[18] In conditioning regimens, it can be used at myeloablative or reduced-intensity dosages, with or without other agents.
- ***Thiotepa***: Thiotepa is an alkylating agent that causes inter-strand crosslinks in DNA, inhibiting DNA and RNA synthesis and causing programmed cell death.[19] It was recently designated for use in conditioning regimens and in high doses is myeloablative.
- ***BEAM regimen (carmustine (BCNU), etoposide, cytarabine, melphalan)*:** The BEAM regimen is a combination therapy that has fewer side-effects than widely used cyclophosphamide/total body irradiation regimens. The BEAM regimen alone may not be sufficiently immunosuppressive to allow allogeneic stem cell engraftment and is used preferentially in autologous HSCT to treat lymphoma. If combined with low-dose total body irradiation, fludarabine or antithymocyte antibody therapy, it can be considered for allogeneic transplantation for patients with lymphoma. ***Total body irradiation (TBI)***: As the name suggests, total body irradiation is the exposure of the entire body to ionizing radiation, with the goal to destroy bone marrow and cancer cells.[20] Typically, the full dose of radiation is divided into smaller doses (fractionated) given over several days, to reduce toxicity and increase tolerability. It is commonly combined with chemotherapy agents, including cyclophosphamide, etoposide, or cytarabine. Major side-effects include mucositis, lung toxicity, and female infertility.
- ***Total lymphoid irradiation (TLI)***: Total lymphoid irradiation is the selective exposure of all major lymph nodes, thymus, and spleen to ionizing radiation, while the non-lymphoid organs are shielded. It causes a potent and long-lasting immunosuppression due to reduced total lymphocytes and T cells, with fewer of the severe side effects associated with total body irradiation.

Conditioning regimens can be broadly categorized into three groups by their ability to remove bone marrow: *myeloablative*, *non-myeloablative*, and *reduced intensity* regimens.[7] There is interest in comparing the effectiveness and safety of these groups to determine which may provide the greatest balance of benefits and harms for patients. The groups are described as follows:

- ***Myeloablative***: Myeloablative regimens involve the administration of TBI as well as alkylating agents (e.g., melphalan, cyclophosphamide), such that hematologic recovery cannot occur. The combinations of BU/CY and CY/TBI are both classified as myeloablative conditioning regimens. Other pharmaceuticals belonging to the myeloablative grouping include etoposide, thiotepa and melphalan. Myeloablative regimens can be associated with high transplant-related mortality,

which is influenced by factors such as patient and donor ages, severity of disease, patient co-morbidities and HLA matching.

- *Working definition: A combination of agents expected to produce profound pancytopenia (i.e. large reduction in the numbers of platelets, red blood cells and white blood cells) and myeloablation (i.e. large reduction of bone marrow activity, also associated with a reduction in the numbers of platelets, red blood cells and white blood cells) within 1–3 weeks of administration; pancytopenia is long lasting, usually irreversible and in most instances fatal, unless hematopoiesis is restored by hematopoietic stem cell infusion.*

- ***Non-myeloablative***: Non-myeloablative regimens were introduced to reduce toxicity relative to myeloablative regimens, allowing patients of greater age and/or increased medical fragility to undergo HSCT. These regimens cause minimal cytopenia (i.e. reduction of blood cells), but have sufficient immunosuppressive effect that full engraftment usually occurs following transplant of allogeneic blood stem cells. This class of regimens is commonly described as being "immunoablative" due to their strong immunosuppressive effect. Transplant-related mortality is typically reduced with this category of regimens in comparison with myeloablative regimens.
  - *Working definition: A regimen that will cause minimal cytopenia and does not require stem cell support.*

- ***Reduced intensity regimens***: Reduced-intensity regimens are considered an intermediate category of conditioning regimens that do not fit the definition of myeloablative or non-myeloablative. They differ from myeloablative regimens in that doses of TBI and alkylating agent are both reduced by a minimum of 30% and transplant-related mortality is reduced. They differ from non-myeloablative regimens in that they cause significant pancytopenia and require stem cell support. That said, hematopoietic recovery without allogeneic transplantation may be possible eventually, but the time required would result in significant morbidity and mortality due to prolonged myelosuppression and profound immunosuppression. Fludarbine is commonly used in combination with either an alkylating agent at a reduced dose (e.g., busulfan, melphalan or thiotepa) or reduced dose TBI.
  - *Working definition: a regimen that cannot be classified as myeloablative or non-myeloablative.*

2.3 Why is it important to do this review?

Numerous agents are used in a broad variety of conditioning regimens for patients undergoing allogeneic HSCT. Additionally, considerable variation in practice exists between institutions, given the lack of comparative evidence to support these interventions, with head-to-head data lacking for many comparisons of possible treatments. A systematic review of the evidence, incorporating network meta-analyses to compare regimens and their impact on key outcomes, will help to explore the relative benefits and harms of competing interventions. Identifying regimens that best balance benefits and harms will improve patient outcomes.

2.4 Existing Literature of Relevance

Initial conditioning regimens were largely radiation based[21] and evolved towards combinations of cyclophosphamide and radiation[22] with small randomized trials emerging in the 1990s to compare transplant outcomes in leukemia.[23] A recent review[8] contrasts and compares the use of common regimens used in allogeneic transplant today, emphasizing the role of reduced-intensity regimens and discussing the selection of high dose chemo-radiotherapy versus chemotherapy alone. Through recent workshops[7,24], the international transplant community has developed definitions and working criteria to describe the intensity of conditioning regimens. Only a small number of regimens have been compared in randomized trials and many regimens are used preferentially to treat specific diseases or in particular patient groups.

2.5 Objective of this Systematic Review

1) To compare the benefits and harms of competing conditioning regimens in patients undergoing HSCT to establish a hierarchy of intervention strategies according to their efficacy and safety.

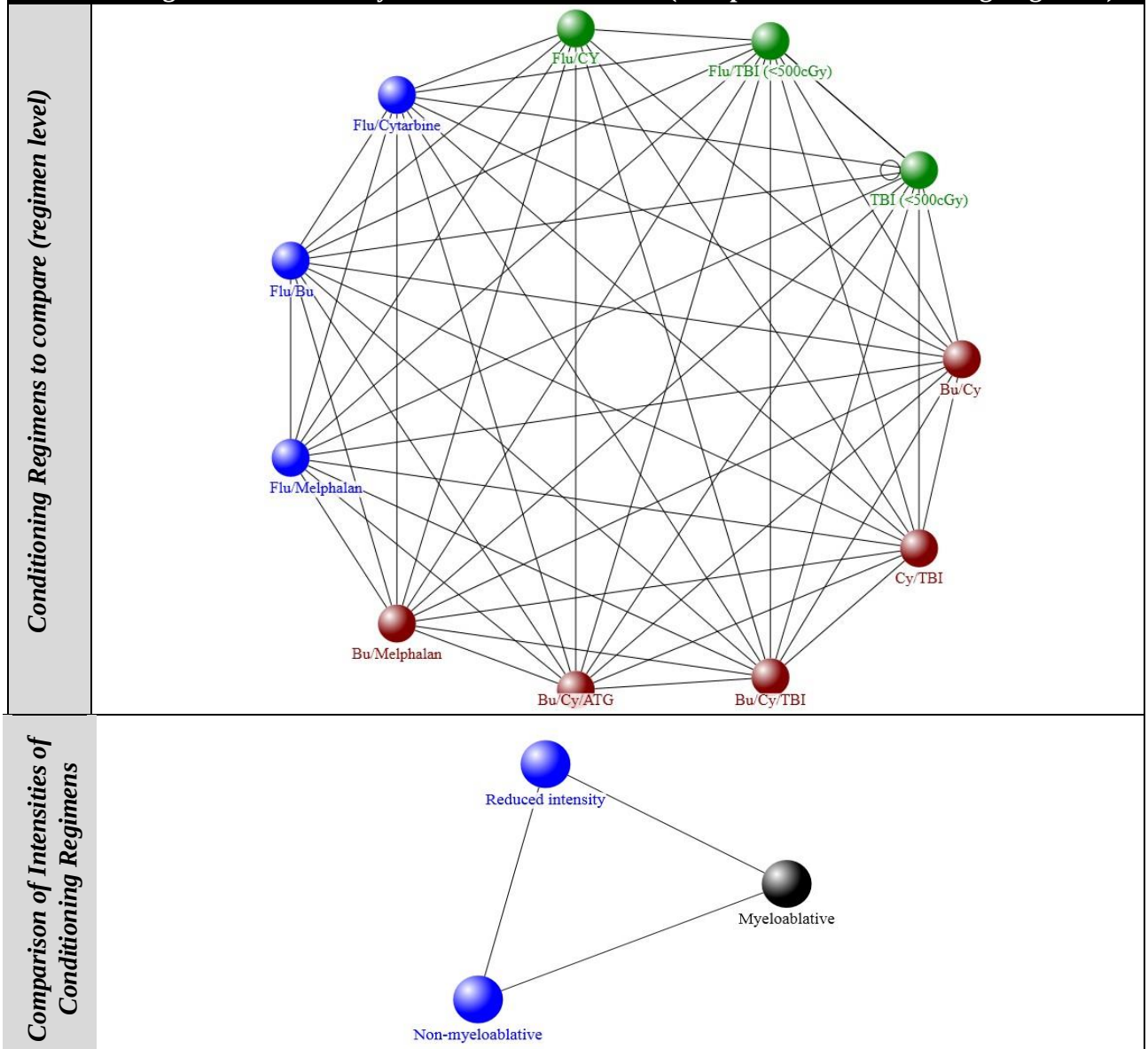
2.6 Methods

2.6.1 Study Eligibility Criteria

Table 1, Eligibility Criteria: Studies to Inform a Comparison of Conditioning Regimens for allogeneic HSCT	
Population	• Patients undergoing allogeneic HSCT for the treatment of Acute Lymphoblastic Leukemia (ALL), Non-Hodgkins Lymphoma (NHL), Chronic Myelogenous Leukemia (CML), Myelodysplastic Syndromes (MDS), Acute Myeloid Leukemia (AML), Multiple Myeloma (MM), Aplastic Anemia (AA), or other cancers.
Intervention and Comparators	• Regimens involving combinations of cyclophosphamide, busulfan, melphalan, fludarabine, total body irradiation, etoposide, BEAM, ATG, alemtuzamab and lymphoid radiation will be included. Myeloablative, non-myeloablative, and reduced intensity regimens will all be eligible. **Figure 1** presents a preliminary network diagram of anticipated combinations used in practice; additional regimens will be incorporated as needed following identification of eligible studies. • As there is no definitive source of relevant doses of these interventions to consider for comparisons, studies involving any dose of agents will be retained during study identification, and we will review intervention details with clinical experts to determine necessary dose stratifications and/or exclusions of specific studies involving irrelevant regimens.
Outcomes	• Survival • All-cause mortality • Transplant-related mortality • Relapse of underlying disease • Incidence of acute GVHD (grade II-IV) • Incidence of chronic GVHD • Veno-occlusive disease (known also as sinusoidal obstruction syndrome)
Study Design	• Randomized controlled trials with at least 100 days of patient follow-up following transplant.

- ***Dealing with duplicate publications and other characteristics.*** For studies that are associated with multiple publications (e.g., updates of different follow-up durations), we will retain the most up-to-date reports and make note of all related manuscripts. Only studies published in English will be retained for inclusion.[25]
- ***Potential for grouping of eligible interventions in the treatment network.*** The following comparisons of and/or groupings of treatments will be considered in potential sensitivity analyses with regard to constructing the geometry of the treatment networks and how interventions may be grouped if deemed helpful by clinical experts:, low-dose radiation regimens (<500 cGy) compared with high dose radiation regimens (>500 cGy), antibody-containing regimens compared with similar regimens without antibody therapy such as ATG, and fludarabine-containing non-myeloablative regimens. We also intend to explore the feasibility of a sensitivity analysis wherein conditioning regimens from the included studies will be categorized according to intensity of the regimen (see **Figure 1**). Preliminary classification of anticipated regimens to be identified is outlined in **Table 2**; if necessary, additional regimens will be classified and incorporated.

Figure 1:
Network Diagram of Preliminary Interventions to Include (Comparison of Conditioning Regimens)



Preliminary regimens for network meta-analysis are shown; the final set of regimens included will depend upon interventions that are studied in RCTs and views of clinical experts as to the relevance of regimens identified. Efforts to separate dose groups will be determined with expert input. ***Abbreviations***: Cy=cyclophosphamide; Bu=busulfan; TBI=total body irradiation; ATG=anti-thymocyte globulin; Flu=fludarbine.

Table 2: Classification Scheme for Conditioning Regimens

Myeloablative Regimens	Non-Myeloablative Regimens	Reduced Intensity Regimens
• Cy/TBI • Bu/Cy/TBI • Bu/Cy • Bu/Cy/ATG • Bu/Melphalan	• Flu/Cy ± ATG • Flu /AraC/ Ida • Flu/TBI (≤500cGy) • TBI (≤500cGy) • Cladribine + AraC • Lymphoid Irradiation/ATG	• Flu/Melphalan • Flu/Bu • Flu/cytarbine

Abbreviations: Cy=cyclophosphamide; Bu=busulfan; TBI=total body irradiation; ATG=anti-thymocyte globulin; Flu=fludarbine; AraC=cytosine arabinoside

2.6.2 Approach to Literature Search

In June 2013, requestors of this DSEN query conducted preliminary work with members of the MAGIC team based at The Ottawa Hospital to explore some of the literature available for developing clinical guidance related to allogeneic HSCT. This was conducted in the form of a scoping review of published RCTs, and involved a systematic search for studies for various aspects of care in the realm of HSCT. The search was developed and conducted with the input of an information specialist and covered the following databases: Medline, PubMed, Embase, and the Cochrane Register of Controlled Trials. The search was also peer reviewed by a second information specialist using PRESS criteria.[26] The search strategy is provided in **Appendix 1**, along with a flow diagram summarizing results from screening.

Following screening of abstracts and then potentially relevant full text reports by two independent researchers, approximately 700 RCTs were identified in relation to the following aspects of care: 1) donor selection and source of cells, 2) conditioning regimens, 3) prevention and treatment of GVHD, 4) transfusion-related interventions, 5) prevention and treatment of infections, 6) prevention and treatment of hepatic sinusoidal obstruction syndrome, 7) prevention and/or treatment of bronchiolitis obliterans, and 8) others that were not otherwise classifiable. There were approximately 50 RCTs that were identified as relevant to the comparison of conditioning regimens. We recently updated the search to identify new studies published since June 2013 for inclusion in the proposed review, producing a total of approximately 2,000 additional citations for review, and we will combine newly found studies with those from the initial search to establish our evidence base. We estimate an additional 20 RCTs for inclusion, resulting in a total of 70 RCTs for this review.

2.6.3 Study Selection Process

For new citations obtained from the updated search, review of citations based on title, keywords, and abstract (Level 1 screening) and full text articles (Level 2 screening) will be carried out independently by two reviewers. Level 1 citations deemed potentially relevant or lacking sufficient information to make a decision will be carried forward to Level 2. Study selection will be conducted using Distiller Systematic Review Software (DSR) (Evidence Partners, Inc; Ottawa, Canada). Where consensus is not achieved following discussion, a third independent party will be consulted to settle disagreements. At both stages of screening, a pilot exercise of a number of abstracts/full texts will be performed to establish a baseline amongst the reviewers. The process of literature selection will be reported using a flow diagram as recommended by the PRISMA statement,[27] and will encompass both the 2013 and 2015 searches performed. Studies will not be screened on outcome; however, studies included at the full-text stage that do not have an outcome of interest to the review will not move forward for data extraction. The citations for these studies will be presented in an appendix of the final report.

2.6.4 Data Collection and Risk of Bias Appraisal

Primary data collection of included studies will be performed independently by two reviewers using a standardized electronic data collection form in DSR. Collected data will be compared for accuracy and agreement, with disagreements being settled by discussion. The following elements will be collected for each included study: study characteristics (*authors, year of publication, journal, countries of performance*), patient characteristics (*eligibility criteria, number per group, and key demographics including age, gender, race, primary diagnosis, disease duration, comorbidities, number of prior treatments received, HLA and CMV matching, and so forth*), interventions (*drug(s) and radiation used as well as dosage, intensity category (reduced intensity, myeloablative or non-myeloablative*) *and other aspects of administration noted within each study*), and outcomes (as mentioned earlier, with number of events and number of patients randomized for binary endpoints, and means with standard deviations for continuous endpoints). All study characteristics will be summarized in tabular form to facilitate inspection and discussion with clinical experts in terms of study heterogeneity, grouping of interventions, and other such topics required to inform analysis; these tables will also be included in our final review.

For assessing risk of bias (RoB), all relevant RCTs will be evaluated using the revised Cochrane RoB tool.[28] The Cochrane RoB tool evaluates seven domains including sequence generation, allocation

concealment, blinding, missing outcome data, selective outcome reporting, attrition, and "other sources of bias." Any disagreements will be resolved through discussion or by third-party adjudication. Results from these appraisals will be summarized in the review and provided in full in an appendix. They will also be considered as criteria for sensitivity analyses.

2.6.5 Review of Study Characteristics to Judge Appropriateness of Homogeneity/Similarity Assumption

An important step in the practice of systematic reviews that incorporate network meta-analyses is the validation of the assumption that patients in the included trials are 'jointly randomizable,' or in other words, that they are sufficiently homogeneous clinically that a patient in any one of the studies could have been a patient in any of the other included trials.[29] We will empirically evaluate this assumption by review of the patient eligibility criteria and pertinent patient demographics, in collaboration with our participating clinical experts and other members of the research team.

To assess the presence of clinical and methodologic heterogeneity within each pairwise comparison of the treatment network, we will inspect trial and population characteristics. This will be performed by inspection of tabulated lists and boxplots of descriptive statistics for the a priori characteristics mentioned below (i.e., means and frequency distributions as appropriate for each characteristic), as well as review of measures of statistical heterogeneity (using the I^2 statistic to identify syntheses with a value >50%, equivalent to moderate or greater heterogeneity).

To ensure transitivity across pairwise comparisons in the treatment network, we will subsequently compare these descriptive statistics of key measures across the different pairwise comparisons in the network to verify they are comparable. To identify covariates necessary for review, we consulted clinical experts and grouped traits that have been identified in past studies of prognostic risk factors.[30-33] The following characteristics will be considered most important to the establishment of transitivity within and across pairwise comparisons:

- Average patient age, gender distribution, and race distribution;
- % with different diagnoses (AML, ALL, CML, etc);
- Average disease duration;
- Measures of disease status (e.g. standard/high risk, Karnofsky performance score, etc);
- Presence of comorbidities (pulmonary disease, rheumatologic disease, renal dysfunction, etc);
- Year of study publication (for consideration of changes in co-interventions such as newer antimicrobials, molecular screening tests for infection, and newer agents used to prevent GVHD);
- % of patients receiving an unrelated donor transplant (including umbilical cord blood);
- % of patients with HLA mismatch (fully matched, one mismatch, more than one mismatch);
- % of patients with mismatched donor gender;
- % of patients that were CMV-seronegative and received transplants from CMV-seropositive donors;
- source of donor cells (bone marrow, peripheral blood stem cells, umbilical cord blood).

We will describe any concerns related to the extent to which included studies meet the transitivity assumption within the final report.

2.6.6 Plans for Evidence Synthesis

2.6.6.1 Interventions to be Compared Using Meta-Analysis

There is considerable variation in institutional practice in the selection of conditioning regimens for allogeneic HSCT. While our literature search will not be limited with regard to interventions sought, the analyses we will undertake in this review will be limited to interventions where at least two eligible RCTs with a minimum of 25 patients per group are available. In setting this criterion, our objective is to generate a more reasonably sized and clinically relevant network of treatments for analysis, as the inclusion of many comparisons that are informed by single studies can be problematic from an analytic perspective and may unnecessarily include conditioning regimens of little or no clinical interest. In addition, prior to data extraction, we will present to our clinical experts the list of interventions compared within potentially eligible trials, in order to exclude studies evaluating regimens that are no longer clinically relevant. All studies that are removed for either of the above rationale will be documented in an appendix in the final report.

2.6.6.2 Approach to Synthesis

We will first conduct traditional meta-analyses of all pairwise comparisons in the network to establish the available direct evidence and the corresponding degree of statistical heterogeneity. Network meta-analyses[34-36] will subsequently be performed if the assumption of transitivity in the treatment network as described earlier is judged to be met.

2.6.6.3 Synthesis of Available Direct Evidence

Standard pairwise meta-analyses will be conducted using random-effects models in Comprehensive Meta-Analyst software (Biostat Inc; Englewood, New Jersey, USA) to generate summary estimates and to assess statistical heterogeneity. All summary estimates will be reported as odds ratios for binary outcomes, mean differences for continuous outcomes, and hazard ratios for time-to-event outcomes, with corresponding 95% confidence intervals. All measures of I^2 will be reported as proportions ranging from 0–100%. I^2 values of 50% or higher will be considered indicative of potentially important heterogeneity which will be explored using established methods such as subgroup analysis, meta-regression and/or exclusion of outlier studies. If necessary, similar approaches will be conducted in network meta-analyses as well to address existing heterogeneity.

2.6.6.4 Synthesis of All Available Evidence (Network Meta-Analysis)

Network meta-analyses will be carried out separately for each of the clinical outcomes of interest. Network meta-analysis is an approach to evidence synthesis that allows for the combination of both *direct* and *indirect* evidence to compare three or more treatments in a unified analysis.[34] Indirect comparisons between treatments A and B based on a common comparator C where no trials of A versus B exist (i.e. no direct evidence) but trials of A versus C and B versus C exist (i.e. indirect evidence) were originally proposed by Bucher et al,[37] and Lumley[38] and Lu and Ades[36] subsequently developed extensions of this methodology. In addition to estimating all possible pairwise comparisons in a network (e.g. summary odds ratios and mean differences), this technique can also be used to estimate probabilities of treatment superiority to rank the treatments including Surface Under the Cumulative Ranking curve (i.e., SUCRA) or median treatment rankings with corresponding 95% credible intervals;[39] these will also be reported as secondary information to help with interpretation of pairwise comparisons. Additional considerations of the approach to the network meta-analysis are as follows:

- **Addressing clinical & methodologic heterogeneity**: Primary analyses will be unadjusted; however, additional analyses to assess heterogeneity will be pursued. We will consider additional analyses to address study deficiencies found in risk-of-bias assessments by using meta-regression (depending on the number of included studies) and/or exclusion of low quality studies. Sensitivity analyses using meta-regression and/or reduction of the treatment network to address clinically important variations between studies with regard to gender distribution, age distribution, indication and other relevant factors noted earlier for establishment of homogeneity will also be pursued. Tables and figures will be used to show the extent of deviations observed from these analyses in comparison to primary analyses carried out.
- **Clinical subgroup analyses**: specific clinical subgroups of interest in this work will include those defined by underlying disease (acute myeloid leukemia and myelodysplastic syndromes, acute lymphoblastic leukemia, chronic leukemias and lymphomas, aplastic anemia and non-malignant diseasesothers). Undertaking a primary analysis of all patients followed by secondary analyses focused to groupings of these indications, as feasible based on available data, will be explored.
- **Reporting of findings from analysis**: full graphical and numeric presentations of findings along with a lay-person's summary will be provided. This will include the following: network diagrams showing the availability of evidence for all possible treatment comparisons; summary odds ratios (or mean differences as appropriate), with 95% credible intervals for all pairwise comparisons between regimens; SUCRA and median treatment rankings (with corresponding 95% CrI) for each outcome. These will be described using approaches recommended by Salanti et al.[39] We will use the checklist of the fortcoming extension of the PRISMA Statement for Network Meta-Analysis (to be published in June 2015) to ensure all findings are clearly reported.[40]
- **Assessment of consistency of direct and indirect evidence**: The treatment networks in this review will consist of a mixture of both direct evidence and indirect evidence. As described by Dias et al. and by Salanti et al. in separate publications, there is a need to verify that the findings generated by direct and indirect data do not differ more than one might expect by chance, such that the validity of combining both types of data is questionable. We will employ statistical methods described by Dias et al.[41] to assess the validity of this assumption for all analyses in this review. Specifically, we will (i) fit inconsistency network meta-analysis models and compare the devaiance information criteria with the corresponding consistency models; and (ii) review scatterplots of the residuals from these models. As statistics alone cannot always be relied upon to identify important clinical or methodologic differences between studies, we will also employ evidence tables of study characteristics to further assess homogeneity. This step will also be important when gathering studies and their data from existing reviews to ensure that all data are relevant to this project.

All network meta-analyses will be performed using WinBUGS software version 1.4.3 (MRC Biostatistics Unit), along with the Microsoft Excel plug-in tool NetMetaXL[42] (for continuous and binary outcomes) to organize all data sets and generate all summary figures including network diagrams, forest plots, league tables and rankograms. Approaches used for these analyses will all follow existing recommendations for modeling of unadjusted and adjusted models as outlined by guidance from experts at the National Institute for Clinical Excellence.[41,43,44] For events expressed in terms of hazard ratios, methods outlined by Woods et al will be employed.[45] Both fixed and random effects models will be fit for each outcome. The fit of a model will be assessed by comparing its posterior residual deviance with the number of unconstrained data points (i.e. the number of intervention arms across all studies) for the analysis. Selection between different models will be based upon deviance information criteria (DIC) for each competing model, with a difference of 5 or more points to be considered significant. Totals of 50,000 or more burn-in iterations and 50,000 or more sampling iterations will be used for all network meta-analyses, and model convergence will be assessed based on inspection of history plots and the Monte Carlo error of all parameters.

2.7 Knowledge Translation Plans

In working with the CBMTG and pCODR on this review, we intend to provide appropriately detailed reports to both organizations to inform future clinical guidance and/or changes in practice that may subsequently be pursued. Sub-committees of the CBMTG will use the data from our analysis to formulate best practices and inform member transplant centres of recommendations and guidelines. Moreover, the clinical trials network of the CBMTG will use the information to plan and execute future studies to generate new knowledge and improve care for patients undergoing allogeneic HSCT. We will also present this information at meetings to both groups as needed for dissemination of findings. Key representatives from our knowledge users will also participate throughout our review process as suggested for an integrated KT approach; they will have the opportunity to provide input at various key landmarks during the review process, including review of study characteristics and discussion of preliminary findings from our analyses. We will also share the findings from our work with DSEN once the review is complete. One or more peer-reviewed publications will be pursued, dependent upon the volume of final results that are generated from our work; we will also publish the protocol and register it with the PROSPERO database of systematic reviews. Submissions for oral presentations at relevant conferences and to relevant stakeholders will be planned.

RESEARCH QUESTION 2:
Comparison of Interventions for Prophylaxis and Treatment of Graft Versus Host Disease (GVHD) in Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplantation

3. Research Question 2: What are the Relative Effectiveness and Harms of Interventions for the Prophylaxis and Treatment of Graft-Versus-Host Disease?

3.1 Description of the condition

Graft-versus-host disease (GVHD) is a complication that can occur after an allogeneic transplant, caused by immune cells in the graft recognize the transplant recipient to be foreign, and subsequently proceed to attack the recipient's cells. Three requirements are necessary for the development of GVHD: (1) the donor transplant must contain immuno-competent cells (T cells); (2) the recipient must express tissue antigens that are foreign to the transplant donor; (3) the recipient must not be able to mount an immune response to eliminate the donor T cells. The first and last of these requirements are met with any allogeneic HSCT. The second requirement, recipient tissue antigens that are foreign to the transplant donor, can be somewhat controlled through careful selection of donors with matched antigens. The most immuno-stimulating of the antigens found on recipient cells are human leukocyte antigens (HLAs). These proteins, also known as the major histocompatibility complex, have high genetic variability, making exact matches between donor and recipient difficult. Despite being fully matched with their donors for the 8 major HLAs, 35–40% of HSCT recipients will develop acute GVHD due to unmatched minor histocompatibility antigens.

Recipients of HSCT are often highly immunosuppressed due to the effects of the pre-transplant conditioning regimen as well as post-transplant management. As a result, they are incapable of mounting an immune response to donor cells, which is beneficial for graft survival, but detrimental when donor T cells attack recipient cells. Myeloablative conditioning regimens are associated with a high risk of acute GVHD (aGVHD) in the first 100 days post-transplant due to their extreme immunosuppressive effects coupled with the important degree of tissue damage and subsequent widespread inflammation. Non-myeloablative techniques reduce the risk of aGVHD in the first 100 days; however, the myeloablative consequences of the graft-versus-tumour effect may simply delay the signs of aGVHD to >100 days post-transplant. Originally, aGVHD was defined as having onset before day 100 post-transplant, with chronic GVHD (cGVHD) arising beyond 100 days. However, this definition may no longer be accurate, as non-myeloablative conditioning regimens gain popularity and late-onset aGVHD and overlap syndrome have become more commonly recognized. Current thought is that clinical manifestations should be used to differentiate aGVHD from cGVHD rather than time of onset.

Classically, patients with aGVHD present with maculopapular rash, nausea, vomiting, anorexia, profuse diarrhea, ileus, or cholestatic hepatitis. The severity of aGVHD can be graded on a scale from I to IV, with higher grade suggestive of greater severity. Patients with Grades III or IV aGVHD have reduced 5-year survival (25% and 5%, respectively).

To differentiate cGVHD from aGVHD, diagnostic signs have been identified. These signs may occur in the skin and appendages, mouth, eyes, female genitalia, esophagus, lungs, or connective tissues. Biopsy may help to confirm GVHD in clinically unclear cases. Chronic GVHD can be manifested in a single organ or may be widespread, leading to debilitating consequences (e.g., joint contractures, loss of sight, end-stage lung disease, or mortality resulting from profound immunosuppression). A 3-level scoring system of cGVHD severity has been proposed (mild, moderate, severe), reflecting the clinical impact of cGVHD on patient function. Incidence rates of cGVHD can reach as high as 80% after HSCT.

3.2 Description of the Interventions and How They Work

A variety of pharmacologic agents are used for prophylaxis and treatment of GVHD in HSCT, many of which have been used for both purposes. Almost all work to suppress donor T cells, with the goal of reducing the risk of GVHD while maintaining sufficient donor T-cell presence to encourage graft-versus-tumour effects. Many immunosuppressive agents have been tried both for prevention and treatment of GVHD, including the following:

- ***Calcineurin inhibitors (cyclosporine, tacrolimus)***: These drugs inhibit calcineurin, a protein involved in the activation of T cells in the immune system.[46] Both cyclosporine and tacrolimus have similar mechanisms of action, clinical effectiveness, and toxic effects. Calcineurin inhibitors are commonly combined with other immunosuppressive drugs (e.g., methotrexate) for prophylaxis.
- ***Mammalian target of rapamycin (mTOR) inhibitors (everolimus, sirolimus)***: mTOR inhibitors prevent the growth and division of cells in the body by blocking mTOR proteins. Specifically, they inhibit T-cell proliferation, when induced by cytokines, including interleukins and colony-stimulating factors.[47]
- ***Corticosteroids***: Corticosteroids suppress a broad range of immune responses mediated by T cells and B cells.[48] T cells may be rapidly depleted from the blood stream due to increased emigration, impairment of growth factor, reduced release from lymphoid tissues, and programmed cell death.
- ***Tyrosine kinase inhibitors***: Tyrosine kinase inhibitors prevent the growth and division of cells in the body by blocking enzymes responsible for sending these signals within the body.[49]
- ***Methotrexate***: Methotrexate is a folate antimetabolite that inhibits DNA synthesis, repair, and replication in actively dividing cells. Methotrexate can reduce antigen-stimulated T-cell proliferation.[50] Common side-effects include neutropenia and mucositis.
- ***Antithymocyte globulin (ATG)***: Antithymocyte globulin dramatically reduces the number of circulating T-lymphocytes through cell lysis. This reduces host immune response in the immediate post-transplant phase, but also increases the risk of infection.[51]
- ***Cyclophosphamide***: Cyclophosphamide is an alkylating agent that causes intra-strand as well as inter-strand DNA crosslinks, preventing DNA replication and causing cell death. It selectively targets certain T cells.[52] Because of high associated toxicity and side-effects, its use is generally of short duration.
- ***Alemtuzumab***: Alemtuzumab is a monoclonal antibody that binds to a protein present on the surface of mature lymphocytes, which are then targeted for destruction.
- ***Mycophenolate mofetil***: Mycophenolate mofetil selectively inhibits the proliferation of T and B lymphocytes through the inhibition of inosine monophosphate dehydrogenase.[53] Mycophenolate mofetil has fewer toxic effects than methotrexate and may encourage more rapid neutrophil engraftment.
- ***Mesenchymal stromal cells.*** Several groups have demonstrated immune modulatory properties of MSCs based on cell-cell contact mechanisms and the secretion of immune bioactive factors.
- ***Hydroxychloroquine (plaquenil)*** and ***extracorporal phototherapy*** have also been studied for use in management of GVHD and will be considered as additional comparators of interest**.**

In clinical practice, while monotherapy remains an option, it is increasingly common for patients to be managed with regimens involving two or more of these interventions. Balancing effectiveness (control of GVHD) with overall safety (no increase in infections or disease relapse) remains a challenge.

3.3 Why is it important to do this review?

Numerous agents are used in a broad variety regimens for both the prophylaxis and treatment of graft versus host disease in HSCT. Additionally, considerable practice variation exists between institutions given the lack of comparative evidence to support these interventions, with head-to-head data lacking for many comparisons of possible treatments. A systematic review of the evidence, incorporating network meta-analyses to compare regimens and their impact on key outcomes, will help to explore the relative benefits and harms of competing interventions in this area.

3.4 Existing Literature of Relevance

While the combination of short course methotrexate combined with calcineurin inhibitor therapy has been the mainstay of GVHD prophylaxsis in most centres for many years, newer approaches that are less toxic

have been investigated, including the use of sirolimus and post-transplant cyclophosphamide to delete allo-reactive T cells. A recent review by Flowers[54] outlines common approaches to treating chronic GVHD using a risk-adapted approach. It is clear that some strategies may work for particular organ involvement but may not work for other organs. A 2014 systematic review compared multiple interventions for prophylaxis of GVHD in patients undergoing allogeneic HSCT,[12] however this review had several limitations including double-counting of several related trials, omission of other relevant trials, and failure to consider several patient-important outcomes including survival, infections, and relapse. Additionally, treatment of GVHD was not addressed.

3.5 Objectives of this Systematic Review

1) To compare the benefits (i.e. prevention of GVHD) and harms (risk of infection and progression or relapse of disease) of competing regimens for prophylaxis of GVHD in patients undergoing HSCT to establish a hierarchy of intervention strategies according to their efficacy and safety.

2) To compare the benefits (i.e. resolution of GVHD, and restoration of organ function) and harms (risk of infection and relapse of disease of competing regimens for treatment of GVHD in patients undergoing HSCT to establish a hierarchy of intervention strategies according to their efficacy and safety.

3.6 Methods

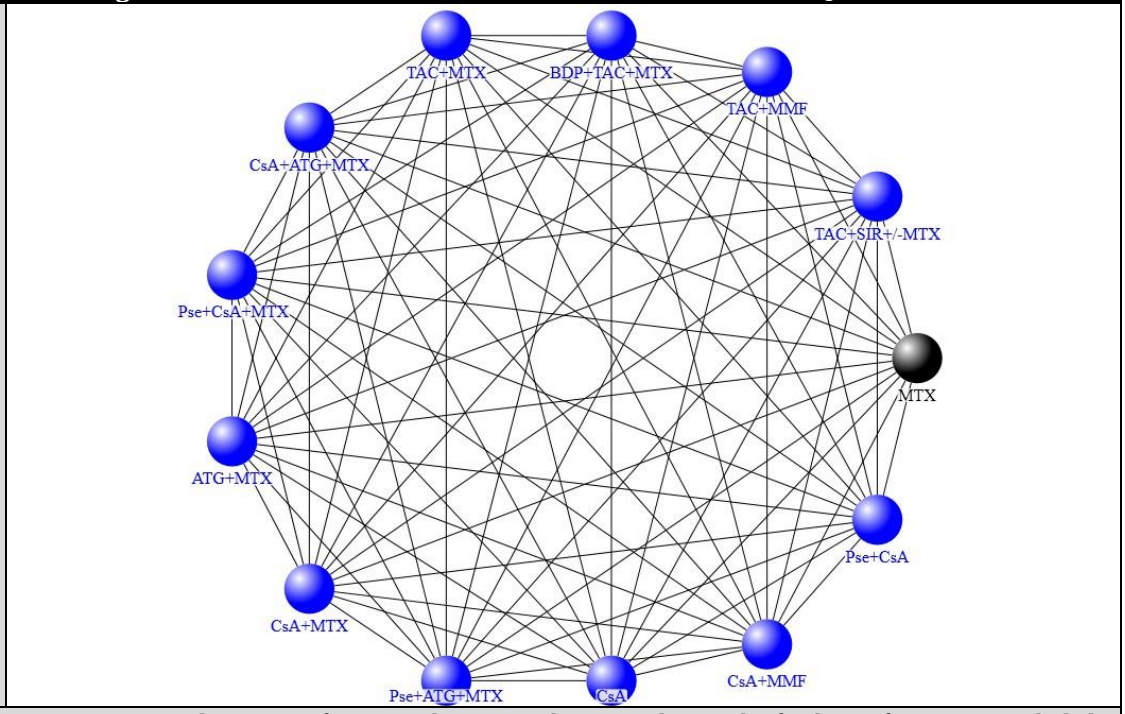
3.6.1 Study Eligibility Criteria

Table 3: Research Question, Prophylaxis of Graft-versus-host Disease	
Population	• Patients undergoing HSCT in the treatment conditions including but not limited to Acute Lymphoblastic Leukemia (ALL), Non-Hodgkins Lymphoma (NHL), Chronic Myelogenous Leukemia (CML), Myelodysplastic Syndromes (MDS), Acute Myeloid Leukemia (AML), Multiple Myeloma (MM) , Aplastic Anemia (AA)
Intervention and Comparators	• Studies of monotherapy and combination therapy involving several agents will be eligible. These include the following: methotrexate, calcineurin inhibitors (tacrolimus, cyclosporin), corticosteroids (prednisolone, methylprednisolone), antithymocyte antiglobulin, and mTOR inhibitors (everolimus, sirolimus), mycofenolate mofetil and cyclophosphamide. • As focused guidance regarding regimens and and drug doses of relevance are not available, inclusion of each regimen will be determined in discussion with our clinical experts as described for Research Question 1.
Outcomes	• Survival • All-cause mortality • Transplantation related mortality • Relapse of underlying disease • Incidence of acute GVHD (within 100 days post-transplant of grades II-IV) • Incidence of chronic GVHD (>100 days post-transplant of grades II-IV) • We will consider late acute GVHD in a sensitivity analysis by including these cases with other occurrences of acute GVHD. Given that overlap syndrome has been defined as an outcome only recently, this outcome will not be studied in meta-analyses. • Specific harms: harms associated with different regimens will be compiled and
Study Design	• Randomized controlled trials of at least 180 days of follow-up

- ***Dealing with duplicate publications and other characteristics.*** For studies that are associated with multiple publications (e.g., updates of different follow-up durations), we will retain the most up-to-date reports and make note of all related manuscripts. Only studies published in English will be retained for inclusion.

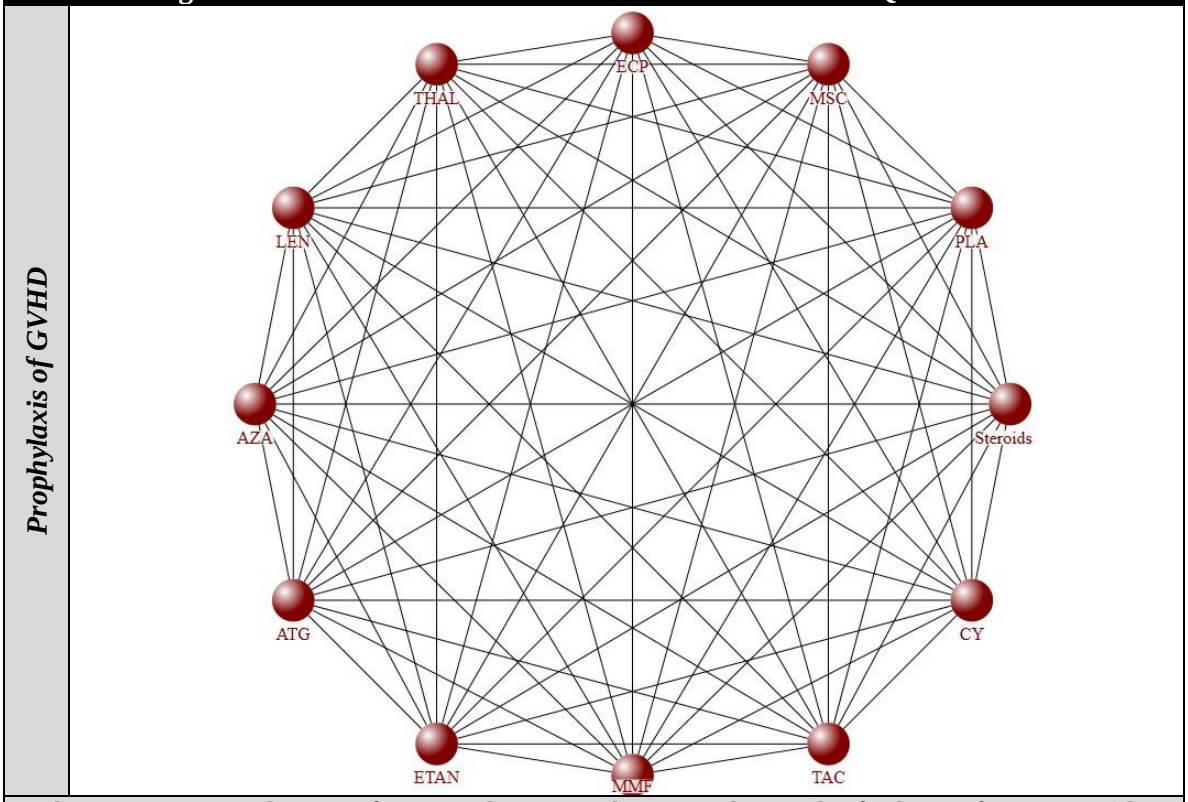
Table 4: Research Question, Treatment of Graft-versus-host Disease	
Population	• Patients undergoing HSCT in the treatment of any condition (as per GVHD prophylaxis question) diagnosed with GVHD after transplantation
Intervention and Comparators	• Studies of monotherapy and combination therapy involving several agents will be eligible. These will include steroids, cyclosporine, tacrolimus, mycophenelate mofetil, etanercept, ATG, azathioprine, lenalidomide, thalidomide, extracorporal phototherapy (ECP), mesenchymal stem cells, and hydroxychloroquine. • Monotherapy and combinations of agents may be identified. **Figure 2** presents a preliminary network diagram of monotherapies, however this network structure will be expanded to include clinically relevant combinations of agents as determined in discussions with clinical participating clinical experts.
Outcomes	• Survival • All-cause mortality • Transplantation related mortality • Resolution of acute and chronic GVHD • Steroid weaning • Weaning from GVHD interventions
Study Design	• Randomized controlled trials of any duration

Figure 2a:
Network Diagram of Interventions to be Included for each Research Question



Preliminary anticipated regimens for network meta-analysis are shown; the final set of regimens included will depend upon interventions that are studied in RCTs and views of clinical experts as to the relevance of regimens identified. Efforts to separate dose groups will be determined with expert input. ***Abbreviations***: CsA=cyclosporine A; MTX=methotrexate; ATG=anti thymocyte globulin; TAC=tacrolimus; MMF=mycophenolate mofetil; SIR=sirolimus; Pse=corticosteroids (e.g. prednisolone).

Figure 2b:
Network Diagram of Interventions to be Included for each Research Question



Preliminary anticipated agents for network meta-analysis are shown; the final set of regimens (also including combination therapies) included will depend upon interventions that are studied in RCTs and views of clinical experts as to the relevance of regimens identified. Efforts to separate dose groups will be determined with expert input. Abbreviations: THAL=thalidomide, LEN=lenalidomide, AZA= azathioprine, ATG=anti thymocyte globulin, ETAN=etanercept, MMF=mycophenolate mofetil, TAC=tacrolimus, PLA=hydroxychloroquine, ECP=extracorporal phototherapy, MSC=mesenchymal stem cells, CY=cyclosporine.

3.6.2 Approach to Literature Search and Study Selection

The results from the scoping search outlined for research question 1 will also provide the evidence base for research question 2. Citation management and screening practices will also be analogous. The 2013 search identified approximately 120 RCTs related to the comparison of interventions for prophylaxis and treatment of GVHD. An additional 20 are expected from the updated 2015 search.

3.6.3 Data Collection and Risk of Bias Appraisal

Primary data collection of included studies will be performed as described for Research Question #1. The following elements will be collected for each included study: study characteristics (authors, year of publication, journal, countries of performance), patient characteristics (eligibility criteria, number per group, and key demographics including age, gender, race, comorbidities, prior treatments received, type of donor and source of cells, HLA matching and CMV serostatus), interventions (radiation and drug(s) used as well as dosage and other aspects of administration noted within each study), and outcomes (as mentioned earlier, with number of events and number of patients randomized for binary endpoints, and means with standard deviations for continuous endpoints). As for research question 1, the Cochrane Risk of Bias scale[28] will be used to appraise the risk of bias of included studies.

3.6.4 Review of Study Characteristics to Judge Appropriateness of Homogeneity/Similarity Assumption

The importance and approach of establishing homogeneity and similarity when pursuing meta-analyses and network meta-analyses was described in the methods for research question 1. An analogous approach will be employed for research question 2 to compare therapies for GVHD, with the following patient demographics being carefully reviewed based on expert input and review of studies that have previously explored predictive risk factors[55-58]:

- Average patient age, gender distribution, and race distribution;
- Year of study publication (for consideration of changes in co-interventions);
- % of patients receiving an unrelated donor transplant;
- % of patients with HLA mismatch (no mismatch, one mismatch, more than one mismatch);
- % of patients with mismatched donor gender;
- % of patients that were CMV-seronegative and received transplants from CMV-seropositive donors;
- Intensity of conditioning regimen used;
- source of cells (bone marrow, peripheral blood stem cells, umbilical cord blood).

3.6.5 Considerations for Evidence Synthesis

3.6.5.1 Interventions to be Compared Using Meta-Analysis

As seen for Research Question 1, while our literature search will not be limited with regard to interventions, the analyses we will undertake in this review will be limited to interventions where at least two eligible RCTs with a minimum of 25 patients per group are available. In setting this criterion, our objective is to generate a more manageably sized and clinically relevant network of treatments for comparison, as the inclusion of many comparisons that are informed by single studies can be problematic analytically. In addition, prior to data extraction, we will also present to our clinical experts the list of interventions compared within potentially eligible trials, in order to exclude studies evaluating regimens that are no longer clinically relevant. All studies that are removed for either of the above rationale will be documented in an appendix of the report.

3.6.5.2 Approach to Synthesis

We will first conduct traditional meta-analyses of all pairwise comparisons in the network to establish the available direct evidence and the corresponding degree of statistical heterogeneity. Network meta-analyses will subsequently be performed.

3.6.5.3 Synthesis of Available Direct Evidence

Standard pairwise meta-analyses will be conducted using random-effects models in Comprehensive Meta-Analyst software to generate summary estimates and to assess statistical heterogeneity. All summary estimates will be reported as odds ratios for binary outcomes and mean differences for continuous outcomes, with corresponding 95% confidence intervals. All measures of I^2 will be reported as proportions ranging from 0–100%. I^2 values of 50% or higher will be considered indicative of potentially important heterogeneity which will be explored using established methods such as subgroup analysis, meta-regression and/or exclusion of outlier studies. If necessary, similar approaches will be conducted in network meta-analyses as well to address existing heterogeneity.

3.6.5.4 Synthesis of All Available Evidence (Network Meta-Analysis)

A general description of the underlying concepts and applications of network meta-analysis was provided earlier for Research Question 1. Additional considerations of the approach to the network meta-analysis are as follows:

- **Addressing clinical & methodologic heterogeneity**: Primary analyses will be unadjusted; however, additional analyses to assess heterogeneity will be pursued. We will consider additional analyses to address study deficiencies found in risk-of-bias assessments by using meta-regression (depending on the number of included studies) and/or exclusion of low quality studies. Sensitivity analyses using meta-regression and/or reduction of the treatment network to address clinically important variations between studies with regard to gender distribution, age distribution, indication (acute leukemia, lymphoma, chronc leukemia)) and other relevant factors will also be pursued. Tables and figures will be used to show the extent of deviations observed from these analyses in comparison to primary analyses carried out.
- **Clinical subgroup analyses**: specific clinical subgroups of interest in this work will include those defined by underlying disease (acute leukemia, lymphoma, chronic leukemia).
- **Reporting of findings from analysis**: full graphical and numeric presentations of findings along with a lay-person's summary will be provided. This will include the following: network diagrams showing the availability of evidence for all possible treatment comparisons; summary odds ratios (or mean differences as appropriate), with 95% credible intervals for all pairwise comparisons between regimens; SUCRA and median treatment rankings (with corresponding 95% CrI) for each outcome. These will be described using approaches recommended by Salanti et al.[39] We will use the checklist of the fortcoming extension of the PRISMA Statement for Network Meta-Analysis (to be published in June 2015) to ensure all findings are clearly reported.[40]
- **Assessment of consistency of direct and indirect evidence**: This review will consist of a mixture of both direct evidence and indirect evidence. As described by Dias et al. and by Salanti et al. in separate publications,[29,41] there is a need to verify that the findings generated by direct and indirect data do not differ more than one might expect by chance, such that the validity of combining both types of data is questionable. We will employ statistical methods described by Dias et al[41] to assess the validity of this assumption for all analyses in this review. As statistics alone cannot always be relied upon to identify important clinical or methodologic differences between studies, we will also employ evidence tables of study characteristics to further assess homogeneity. This step will also be important when gathering studies and their data from existing reviews to ensure that all data are relevant to this project.

All network meta-analyses will be performed using WinBUGS software version 1.4.3 (MRC Biostatistics Unit), along with the Microsoft Excel plug-in tool NetMetaXL[42] (for binary and continuous outcomes) to organize all data sets and generate all summary figures including network diagrams, forest plots, league tables and rankograms. For events expressed in terms of hazard ratios, methods outlined by Woods et al will be employed.[45] Both fixed and random effects models will be fit for each outcome. The fit of a model will be assessed by comparing its posterior residual deviance with the number of unconstrained data points (i.e. the number of intervention arms across all studies) for the analysis. Selection between different models will be based upon deviance information criteria (DIC) for each competing model, with a difference of 5 or more points to be considered significant. Totals of 50,000 or more burn-in iterations and 50,000 or more sampling iterations will be used for all network meta-analyses, and model convergence will be assessed based on inspection of history plots and the Monte Carlo error of all parameters.

3.7 Knowledge Translation Plans

In working with the CBMTG and pCODR on this review, we intend to provide appropriately detailed reports to both organizations to inform future clinical guidance and/or changes in practice that may subsequently be pursued. Subcommittees of the CBMTG will use the data from our analysis to formulate best practices and inform member transplant centres of recommendations and guidelines. Moreover, the clinical trials network of the CBMTG will use the information to plan and execute future studies to generate new knowledge and improve care for patients undergoing allogeneic HSCT. We will also present this information at meetings to both groups as needed for dissemination of findings. Key representatives from our knowledge users will also participate throughout our review process as suggested for an integrated KT approach; they will have the opportunity to provide input at various key landmarks during the review process, including review of study characteristics and discussion of preliminary findings from our analyses. We will also share the findings from our work with DSEN once the review is complete. One or more peer-reviewed publications will be pursued, dependent upon the volume of final results that are generated from our work. Submissions for oral presentations at relevant conferences and to relevant stakeholders will be planned.

RESEARCH QUESTION 3:
Comparison of Interventions for Control of Infections in Patients Undergoing Hematopoietic Stem Cell Transplantation

4. Research Question 3: What are the relative safety and effectiveness of different drugs used to prevent and treat infections, following blood stem cell transplantation?

4.1 Description of the Condition

While HSCT has become a vital therapy in treating patients with a variety of malignant and non-malignant disorders, a challenge that remains is the sizable mortality rate related to infection. Although advances in antimicrobial therapies in HSCT have occurred in recent years, infection still accounts for 16–19% of deaths after allogeneic HSCT.[10] Great variability exists between treatment facilities regarding the care of HSCT patients with respect to infection prevention and treatment. This systematic review will aim to provide evidence to guide best practice development around this area of HSCT patient care.

Ninety percent of infections in HSCT recipients are bacterial in origin.[59] Conditioning regimens prior to stem cell transplant induce cytotoxic effects on epithelial surfaces as well as profound neutropenia. Damage to epithelial surfaces, especially the oral and gastrointestinal mucosae, leads to a series of effects, including the initiation of an inflammatory cascade, mucositis, increased mucosal permeability, and translocation of commensal microorganisms across normally impermeable surfaces.[60] Systemically, profound neutropenia caused by the conditioning regimen prevents a normal immune response to invading commensals, potentially allowing infection to occur. If antimicrobial prophylaxis is not instituted, a potentially life-threatening infection may occur.

Invasion of commensal micro-organisms can occur at any site of reduced epithelial integrity, including the upper and lower respiratory tracts, upper and lower gastrointestinal tracts, and the skin.[60] Injured oral mucosa may allow invasive infections caused by viridans group streptococcus and/or other oral anaerobes such as *Veillonella* spp., or *Fusobacterium* spp., while invaders of gastrointestinal mucosae may include *Escherichia coli, Klebsiella pneumoniae, gut anaerobes such as Clostridium spp.,* or *Bacteroides spp., and opportunistic fungal organisms such as Candida spp.*[60]

From the gastrointestinal system, microorganisms translocate into the blood stream through damaged mucosa and are extracted in the portal circulation by the liver, where they are phagocytized by Kupffer cells (tissue macrophages), stimulating the production of inflammatory cytokines including interleukin-1 (IL-1).[61] IL-1 acts as an endogenous pyrogen, causing fever and the production of acute phase proteins such as fibrinogen. When the capacity of the Kupffer cells to remove the invading bacteria is overwhelmed, sepsis will occur without therapeutic intervention; however, preventive antimicrobial therapy may temper or prevent the systemic inflammatory response to the bacteremia. Despite antimicrobial therapy, pyrexia may persist due to the ongoing production of IL-1 by the Kupffer cells. Fever in the presence of reduced circulating neutrophils is termed febrile neutropenia.

Typically, febrile neutropenia occurs 10–14 days after the first day of the conditioning regimen.[60] This coincides with the timing of the nadir of the circulating absolute neutrophil count, as well as the time of maximal oral and gastrointestinal mucositis, leading to commensal translocation. The timing of occurrence of febrile neutropenia after reduced-intensity conditioning regimens remains the same despite the induction of less profound neutropenia and mucositis.[60] Febrile neutropenia may or may not be associated with a clinical focus of infection; however, pyrexia may be the earliest and only sign of infection in the neutropenic cancer patient, thus warranting rapid workup and empiric systemic antimicrobial therapy.[60]

First-line antimicrobial prophylaxis or therapy in patients with suspected bacterial infection after HSCT usually comprises anti-pseudomonal antibiotics such as a quinolone and/or empiric therapy with anti-pseudomonal or third-generation cephalosporins. Additional agents may be needed depending on antibiotic resistance patterns or other special indications. antibiotic (see the description of the interventions below for more detail).[62,63] Adjustments of antimicrobial agent(s) may be needed based on

an identification of specific microbial organism(s) and its antimicrobial susceptibility profile by laboratory testing, if possible.[59] When culture and susceptibility are not available, subsequent antimicrobial selection depends on suspected source of infection and possible etiologic microbial agents. Often times, this leads to changes in antimicrobial agents to broader coverage of wider range of microorganisms. Antibiotic therapy often reduces protective commensal bacteria, leading to the overgrowth of opportunistic organisms, including fungal infections; thus, antifungal therapy may be required in combination with antibacterial medications.

Viral infections may also cause severe morbidity following HSCT. Cytomegalovirus (CMV) is a human Herpesvirus, with a wide spectrum of disease severity, depending on the immune status of the host. Like all Herpesviruses, CMV has the ability to lie latent, deep in body tissues, through immunosuppressive mechanisms of its own,[64] until a time when host responses are weak and it can reactivate and cause disease. In the immunocompromised HSCT recipient, CMV can cause significant morbidity and mortality, with the natural history of the disease being dependent upon the relationship of the infection status of both donor and recipient.[64] CMV-negative recipients lack acquired immunity to CMV and are especially at risk of developing highly pathogenic infections, if they receive stem cells from a CMV-positive donor.[65] To prevent CMV-related disease, pre-emptive antiviral therapeutics are provided (e.g., ganciclovir, valganciclovir).[64] While the medications may effectively suppress CMV infection, they also effectively prevent development of acquired immunity in the recipient. When drug removal occurs after 6 months or more, CMV load rebound can be expected, and without acquired immunity to control it, severe disease may occur, including pneumonia, hepatitis, and effects throughout the entire gastrointestinal tract.[64] Because of its broad range of severe effects, CMV infection post-HSCT may be confused with widespread GVHD. As well, CMV-negative recipients of CMV-positive stem cells may experience higher mortality due to bacterial and fungal infections than those receiving CMV-negative stem cells, possibly due to the innate immunosuppressive effects of CMV or its therapy.[64]

Compared to CMV-negative HSCT recipients, CMV-positive recipients are at lower risk of developing severe CMV-related disease when transplanted with CMV-positive stem cells. However, due to the immunosuppressive effects of conditioning regimens, latent CMV infection may reactivate.[64] Reactivation is treated pre-emptively, using ganciclovir or valganciclovir, sometimes based on increasing viral load identified by quantitative PCR.[64] Reactivated CMV infections are usually less severe than primary CMV infections, and there is some suggestion that patients with reactivated CMV may be less likely to develop GVHD, potentially due to immunosuppressive effects of the virus itself.

4.1.1 Natural history of infections in HSCT recipients

During the pre-engraftment phase (0–45 days after transplant), prolonged, profound neutropenia and inevitable breaks in mucocutaneous barriers heighten the risk for bacteremia, fungal infections, respiratory viral and reactivation of latent viral infections such as herpes simplex virus (HSV). In the early post-engraftment phase (30 to 100 days after transplant), cell-mediated immunity is impaired, often due to GVHD and concomitant immunosuppressive therapy. Patients continue to be at risk for invasive bacterial infection and have increased risk of other viral infections such as CMV, Epstein-barr virus (EBV), and varicella zoster virus (VZV). During late post-engraftment, the presence of chronic GVHD causes continued susceptibility to infections caused by viruses and encapsulated bacteria such as *Streptococcus pneumoniae*. As well during this period, removal of antiviral drugs may result in rebound CMV disease that may mimic GVHD.

The time since transplantation and the presence of GVHD are the primary risk factors for infection occurring in HSCT recipients; however, other influencing factors include donor/host histocompatibility (HLA mismatched vs. matched; allogeneic vs. autologous/syngeneic), disease severity at the time of transplant, graft type (umbilical cord vs. bone marrow vs. colony-stimulating factor-mobilized peripheral blood stem cells), graft contents (T-cell depletion vs. no T-cell depletion), conditioning intensity, and neutrophil engraftment.[11,62]

4.2 Description of the Interventions and How They Work

A variety of pharmacologic agents are used for the prevention and treatment of different types of infections in patients undergoing HSCT. They include the following:

- **For bacterial infections:**
  - ***Trimethoprim/Sulfamethoxazole***: Trimethoprim/sulphamethoxazole is a combination antibiotic that inhibits folate biosynthesis and metabolism within bacterial cells.[66] It is considered to be bactericidal.
  - ***Fluoroquinolones (e.g., levofloxacin, ciprofloxacin)***: The fluoroquinolones are bacterial DNA gyrase inhibitors and act by preventing the unwinding of DNA for replication.[67] Fluoroquinolones are commonly used to prevent infection 0–100 days after adult HSCT. Their use in children is limited due to associated musculoskeletal adverse effects but can be used in high-risk group i.e. allogeneic transplantation or during induction therapy for acute leukemia. Resistance due to frequent prophylactic use may have limited their utility.
  - ***Cephalosporins*:** Cephalosporins are a group of beta-lactam antibiotics. Beta-lactams inhibit bacterial cell membrane formation, having a bactericidal action. First-generation cephalosporins were mainly active against Gram-positive bacteria; however, subsequent generations have increased activity against Gram-negative organisms, often with a concomitant lower activity against Gram-positive organisms. Some cephalosporins have anti-pseudomonal activity, and thus further classification by generation of cephalosporin will be explored in our analyses if sufficient data is available.
  - ***Penicillin and derivatives (e.g., amoxicillin)***: Penicillins are a group of beta-lactam antibiotics and, thus, have a bactericidal action. Antibiotic resistance is high amongst the penicillins and their derivatives due to beta-lactamase production by bacteria; however, beta-lactamase-resistant penicillins have been developed (e.g., dicloxacillin). Piperacillin-tazobactam is a common agent used as empiric treatment of febrile neutropenia and can overcome beta-lactamase resistance. Further classification by presence of anti-pseudomonal activity will be explored in our analyses if sufficient data is available
  - ***Carbapenems***: Carbapenems are also a group of beta-lactam antibiotics and are highly resistant to most beta-lactamases, inducing broad spectrum antibacterial activity. Carbapenems are considered by some to be antibiotics of last resort, to be used only after all other treatment options have failed. Recent identification of resistance in coliforms due to production of carbapenemases has raised alarm.
  - ***Glycopeptide antibiotics*** (***e.g. vancomycin***): Glycopeptide antibiotics are a group of drugs which inhibit peptidoglycan synthesis to halt the synthesis of cell walls in susceptible microbes. They are often used in more severely ill patients who have been diagnosed as having a β-lactam resistant infection (or who have a hypersensitivity to β-lactams).
  - ***Linezolid:***Linezolid is a bacteriostatic agent, meaning that it halts bacterial growth by interrupting their production of proteins. It is generally used to treat serious infections from gram-positive bacteria which are found to be resistant to other forms of antibiotics, including pneumonia.

- **For fungal infections:**
  - ***Triazole antifungals (e.g., fluconazole, itraconazole, voriconazole, posaconazole)***: The triazole antifungals are antifungal medications that interfere with fungal cell membrane synthesis.[68] Posaconazole has a broader spectrum activity than itraconazole, which is broader than fluconazole. The triazoles have fewer nephrotoxic effects than amphotericin B but many drug interactions.

- ***Amphotericin B***: Amphotericin B is a polyene antifungal agent that disrupts fungal cell wall synthesis.[69] It has a broad spectrum of activity, is considered fungicidal, and is often the standard treatment for severe, invasive fungal infections. Due to its high toxicity, it is usually reserved for life-threatening conditions but is less toxic in its liposomal formulation.
- ***Echinocandin antifungals (e.g., caspofungin, micafungin)***: The echinocandin antifungals target the fungal cell wall, resulting in a fungicidal activity.[70] They are especially active against *Candida* spp. that may be resistant to triazole antifungals, and have low nephro- and hepatotoxicity. Micafungin has been approved by the FDA as a prophylactic agent for *Candida* infections in adults undergoing HSCT.

- **For viral infections:**
  - ***Nucleoside analog antivirals (e.g., acyclovir, valacyclovir, famciclovir)***: The nucleoside analog antivirals act to competitively inhibit viral DNA synthesis in infected host cells. They selectively inhibit herpes simplex virus types 1 and 2 and varicella-zoster virus. Valacyclovir and famiclovir are later generation agents.
  - ***Acyclic analogs of nucleoside guanosine (e.g., ganciclovir, valganciclovir)***: Ganciclovir and its prodrug valganciclovir are antivirals that act to inhibit viral DNA synthesis in infected host cells.[71] These drugs are used primarily for the treatment of cytomegalovirus infections.
  - ***Letermovir***: Letermovir is an investigational antiviral drug for the treatment of cytomegalovirus infections. It is currently under testing and has been granted fast-track status by the FDA. Letermovir is derived from the quinazolines and acts to inhibit human CMV viral terminase.[72]
  - ***Brincidofovir (CMX001):*** Brincidofovir is also an investigational antiviral drug for the treatment of cytomegalovirus. Similar to letermovir, brincidofovir has also been granted fast-track status by the FDA and is being studied in phase III clinical trials. This agent is a prodrug of cidofovir and increases cidofovir activity against dsDNA viruses. It has less adverse effects as compared to cidofovir.

4.3 Why is it important to do this review?

Numerous agents are used for the prophylaxis and treatment of viral, bacterial, and fungal infections at various phases of the HSCT process. Additionally, considerable practice variation exists between institutions given the lack of comparative evidence to support these interventions, with head-to-head data lacking for many comparisons of possible treatments. A systematic review of the evidence, incorporating network meta-analyses to compare these agents and their impact on key outcomes, will help to explore the relative benefits and harms of competing interventions in this area.

4.4 Objectives of this Systematic Review

1) To compare the benefits and harms of competing preventative (includes pre-emptive strategies) and treatment (includes pre-emptive/empiric treatment) agents for **bacterial infections** in patients undergoing HSCT to establish a hierarchy of intervention strategies according to their efficacy and safety.
2) To compare the benefits and harms of competing preventative (includes pre-emptive strategies) and treatment (includes pre-emptive/empiric treatment) agents for **fungal infections** in patients undergoing HSCT to establish a hierarchy of intervention strategies according to their efficacy and safety.
3) To compare the benefits and harms of competing preventative (includes pre-emptive strategies) and treatment (includes pre-emptive/empiric treatment) agents for **viral infections** in patients undergoing HSCT to establish a hierarchy of intervention strategies according to their efficacy and safety.

4.5 Methods

4.5.1 Study Eligibility Criteria

To address the different forms of infection in this systematic review, separate networks of interventions have been established a priori. Each is outlined in the sections which follow below, along with preliminary network diagrams to visualize the competing interventions that will be considered for each form of infection. In all cases, the selection of comparators has been informed by discussion with experts in HSCT and infectious diseases; these structures will be reviewed again with experts following completion of data collection, when study characteristics are discussed. Different network meta-analyses will be considered for prevention and treatment of infections. Preventive medications may be either *prophylactic* (i.e., there is a risk of infection and consequent disease, and medication is given as a prevention) or *pre-emptive* (i.e., an infectious agent may already be present and medication is given to prevent disease). Medications used for *treatment* are given after symptoms and signs of disease have already occurred; they may be given presumptively or empirically, based on symptoms and without evidence of likely efficacy. Where variations exist between networks for prevention versus treatment of the different forms of infection, these distinctions are noted. Key considerations are presented first for prevention and treatment of bacterial infections, and then subsequently for fungal and viral infections. Given important variation in the risk of infections in patients receiving prophylaxis for allogeneic versus autologous transplants, analyses related to prophylaxis will focus on studies involving allogeneic transplants only. For treatment of infection, where transplant type is of lesser consequence in treatment selection, data from patients undergoing both allogeneic and autologous transplants will be included.

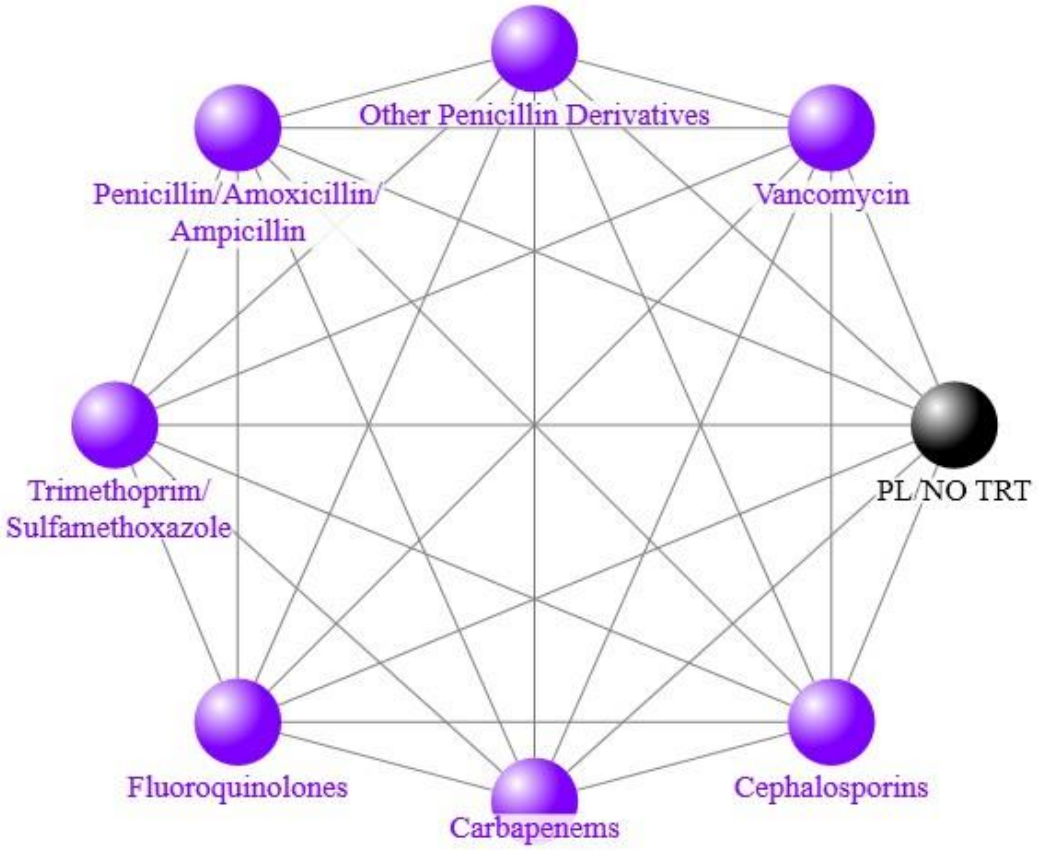
4.5.1.1 Prevention and Treatment of Bacterial Infections

Table 5 outlines PICOS elements for the research questions addressing the benefits of competing interventions for prevention and for treatment of bacterial infections.

Table 5: Research Question, Prophylaxis and Pre-emptive/Empiric Treatment for Bacterial Infections	
Population	• For prophylaxis, patients undergoing allogeneic HSCT as described for research question 1. • For treatment, patients undergoing either allogeneic or autologous HSCT will be of interest.
Intervention and Comparators	• Fluoroquinolones, penicillin and derivatives, cephalosporins, carbapenems, trimethoprim/sulfamethoxazole, glycopeptide antibiotics (vancomycin as well as others), linezolid, placebo/no treatment • No restrictions on treatment dosage will be in place. Both intravenous and oral formats of all agents will be eligible.
Outcomes	• All-cause mortality • Transplant-related mortality • Relapse of underlying disease • % of patients with febrile neutropenia • Incidence of culture-positive bacteremia • Incidence of hospital-acquired pneumonia • Incidence of pneumonia • incidence of multi-drug resistant organisms (e.g. Methicillin resistant S. aureus (MRSA), extended spectrum betalactamase (ESBL) producing organisms, AmpC-producing gram-negative bacilli, multidrug (≥ 2 antipseudomonal) resistant pseudomonas, vancomycin resistant enterococcus (VRE)
Study Design	• Randomized controlled trials

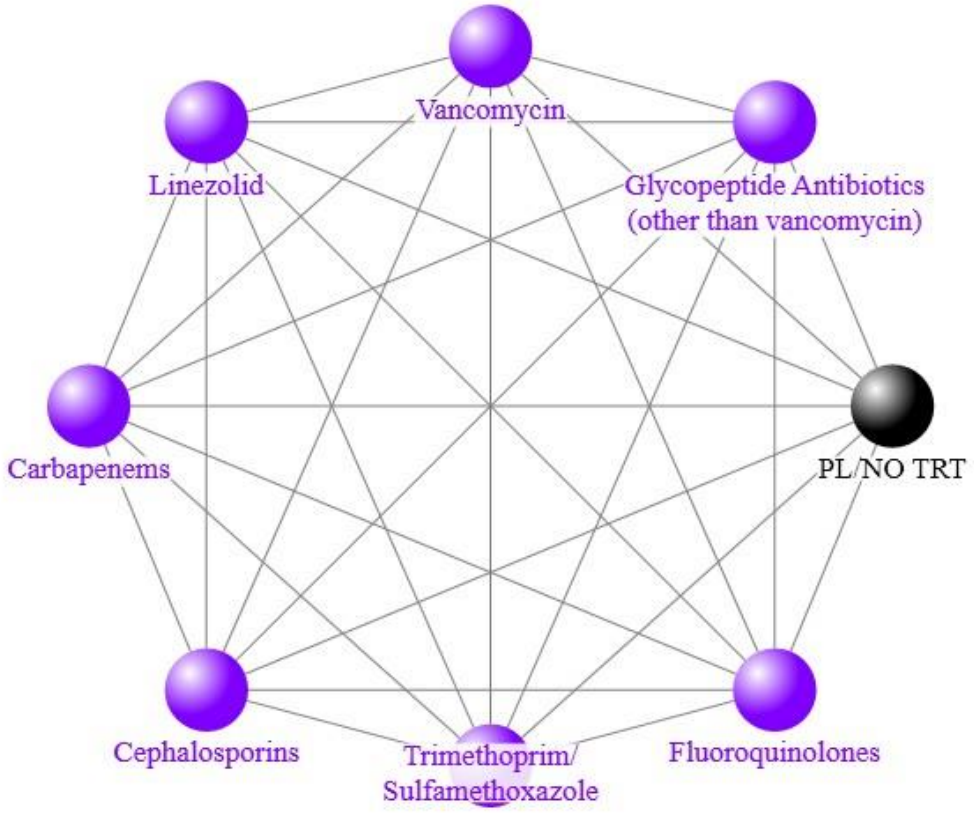
The network diagrams shown in **Figures 3a-3b** present the network structures of interventions to be compared in separate analyses for prevention and treatment purposes for bacterial infections. Clinical expertise suggests that: (1) agents within the presented classes are widely considered interchangeable within each class; and (2) there is extremely broad practice variation in terms of agents and dose ranges used and poor reporting of this information in many of these trials. As such, we anticipate performing network meta-analyses at the class level only. This aspect of the review will be reviewed again with experts after data collection is completed. If sufficient data are available, we will incorporate additional nodes to further refine the network structure and to enable study of the effects of different generations of cephalosporins (generations 1-5) as well as the different penicillins and derivatives in order to study the effects of treatments with anti-pseudomonal activity.

Figure 3a:
Network Diagram of Interventions for Prophylaxis of Bacterial Infections



Preliminary network diagram structure presenting groupings of interventions to be compared using network meta-analysis. The extent to which comparisons of different interventions are supported by randomized trials will vary dependent upon the literature identified and the reporting of outcomes across studies. If sufficient data are available, the classes of cephalosporins and penicillin/derivatives will be further sub-divided to distinguish between those agents with antipseudomonal activity.

Figure 3b:
Network Diagram of Interventions for Treatment of Bacterial Infections



Preliminary network diagram structure presenting groupings of interventions to be compared using network meta-analysis. The extent to which comparisons of different interventions are supported by randomized trials will vary dependent upon the literature identified and the reporting of outcomes across studies. If sufficient data are available, the classes of cephalosporins and penicillin/derivatives will be further sub-divided to distinguish between those agents with antipseudomonal activity (including ceftazidime as separate group).

4.5.1.2 Prevention and Treatment of Fungal Infections

Table 6 outlines PICOS elements for the research question addressing the benefits of competing interventions for prevention and treatment of fungal infections.[73]

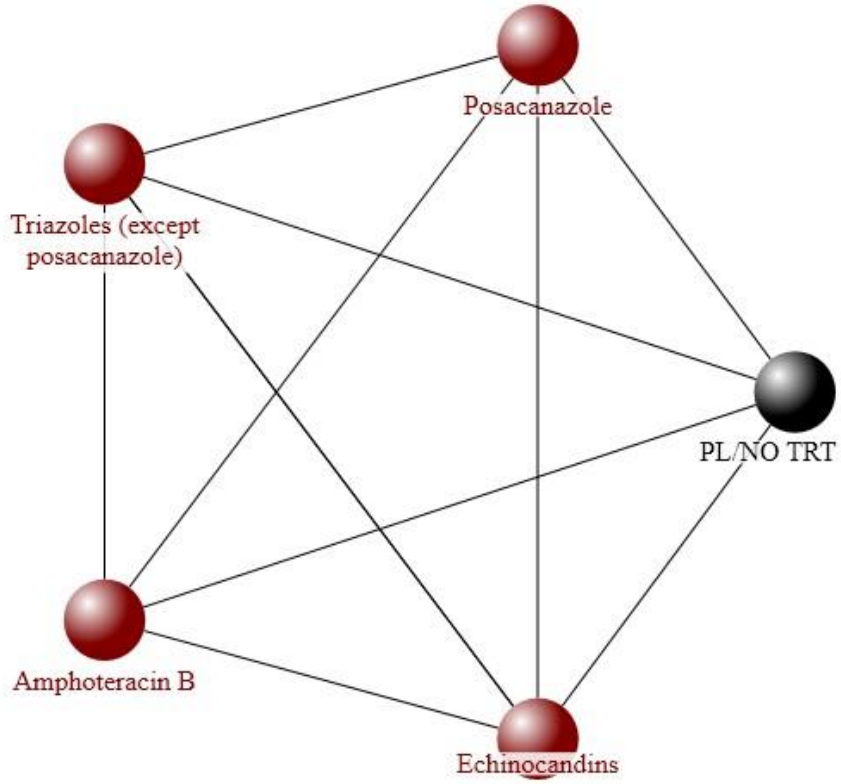
Table 6: Research Question,
Prophylaxis and Pre-emptive/Empiric Treatment for Fungal Infections

Population	• For prophylaxis, patients undergoing allogeneic HSCT as described for research question 1. • For treatment, patients undergoing either allogeneic or autologous HSCT will be of interest.
Intervention and Comparators	• Triazoles except posacanazole (fluconazole, iraconazole/voriconazole), posacanazole, echinocandin anti-fungals, amphoteracin B, placebo/no treatment • no restrictions on treatment dosage will be used. Both intravenous and oral

Table 6: Research Question, Prophylaxis and Pre-emptive/Empiric Treatment for Fungal Infections	
	formats of all agents will be eligible.
Outcomes	• All-cause mortality • Transplant-related mortality • Relapse of underlying disease • % of patients with febrile neutropenia • Incidence of culture-positive bacteremia • Incidence of hospital-acquired pneumonia • Incidence of pneumonia • % of patients with cultures positive for invasive fungal species or testing of pathology specimens confirming presence of invasive fungal infection • Incidence of suspected fungal infection based on imaging but not proven • Incidence of *C. difficile*
Study Design	• Randomized controlled trials

The network diagram shown in **Figure 4** presents the network structure of interventions to be compared in separate analyses for prophylaxis and treatment. Clinical expertise suggests that: (1) agents within the presented classes are widely considered interchangeable within each class (except for triazoles); and (2) there is broad practice variation in terms of agents and dose ranges used and poor reporting of this information in many of these trials. As such, we anticipate performing network meta-analyses at the class level only. This aspect of the review will be reviewed again with experts after data collection.

Figure 4:
Network Diagram of Interventions for Fungal Infections Analyses



Preliminary network diagram structure presenting groupings of interventions to be compared using network meta-analysis. The extent to which comparisons are supported by randomized trials will vary dependent upon the literature identified and the reporting of outcomes across studies.

4.5.1.3 Prevention and Treatment of Viral Infections

Table 6 outlines PICOS elements for the research question addressing the benefits of competing interventions for prevention and treatment of viral infections including cytomegalovirus and other herpesviruses such as HSV or HHV6.

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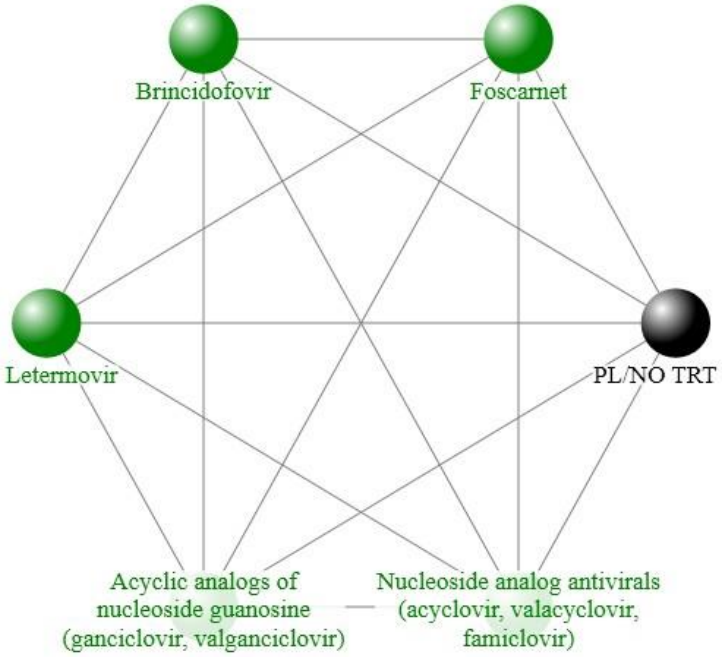
Table 7: Research Question, Prophylaxis and Pre-emptive/Empiric treatment for Viral Infections	
Population	• For prophylaxis, patients undergoing allogeneic HSCT as described for research question 1. • For treatment, patients undergoing either allogeneic or autologous HSCT will be of interest.
Intervention and Comparators	• Nucleoside analog antivirals, acyclic analogs of nucleoside guanosine, letermovir, brincidofovir, Foscarnet, placebo/no treatment • No restrictions on treatment dosage will be used. Both intravenous and oral formats of all agents will be eligible.
Outcomes	• All-cause mortality • Transplant-related mortality • Relapse of underlying disease • % of patients with febrile neutropenia • Incidence of culture-positive bacteremia • Incidence of hospital-acquired pneumonia • Incidence of pneumonia • % of patients with PCR positive CMV reactivation, positive shell vial assay for CMV or positive pathology sample confirming CMV disease • % of patients with HSV serology or PCR confirming presence of active viral infection • % of patients with positive test for EBV or other viral infections (PCR, culture, pathology) • Incidence of acyclovir resistant HSV • Iincidence of ganciclovir/valganciclovir resistant CMV disease, including pneumonia
Study Design	• Randomized controlled trials

The network diagram shown in **Figure 5** presents the network structure of interventions to be compared in separate analyses for prophylactic and treatment purposes. Clinical expertise suggests that: (1) agents within the presented classes are widely considered interchangeable within each class; and (2) there is extremely broad practice variation in terms of agents and dose ranges used and poor reporting of this information in many of these trials. As such, we anticipate performing network meta-analyses at the class level only. This aspect of the review will be reviewed again with experts after data collection is completed.

Figure 5:
Network Diagram of Interventions to be Included for Viral Infections Analyses



Preliminary network diagram structure presenting groupings of interventions to be compared using network meta-analysis. The extent to which comparisons are supported by randomized trials will vary dependent upon the literature identified and the reporting of outcomes across studies.

4.5.1.4 Additional Considerations Related to Treatment Networks

- ***Dealing with duplicate publications and other characteristics.*** For studies that are associated with multiple publications (e.g., updates of different follow-up durations), we will retain the most up-to-date reports and make note of all related manuscripts. Only studies published in English will be retained for inclusion.

- ***Grouping of eligible interventions in the treatment network.*** In our overviews of treatment networks presented above for prevention and treatment of each infection type, we have outlined our approach to grouping of drugs at the class level, as suggested by clinical experts. We have also noted that no a priori dose stratifications were considered worthwhile (and may not be feasible). We will consult with our experts once more after data collection to confirm whether any modifications for our network structures are worthwhile. We will also explore interest in any sensitivity analyses which would distinguish between intravenous and oral forms of the drugs. If combination therapies involving multiple agents are encountered, we will discuss their clinical relevance with team members prior to incorporating them into our analyses, and will continue with classification into our scheme of treatments so as to maximize clinical relevance.

- ***Diagnosis/definition of outcomes***. Classify proven infections based on culture or biopsy results only, including sputum samples, blood samples, brochoalveolar lavage fluids or other tissues.

- ***Outcomes not considered in the planned review***.
    - There are a variety of clinical outcomes of potential interest for clinicians and decision-makers that have been included in this review. However, based on discussion with our clinical experts, certain outcomes have been omitted from this review. For example, while rates of re-hospitalization and length of hospital stay are often of interest, these

outcomes are unlikely to be responsive in change to the interventions being compared in this review. Similarly, in practicality the likelihood of re-hospitalization is likely to be equally influenced by the patient's access (or lack thereof) to relatives and friends to assist with their care upon their return home. While antibiotic resistance remains a large clinical concern, it is also known to be associated with highly variable reporting styles which may limit the ability to conduct meaningful meta-analysis. We will attempt to capture by geographic region of studies in a sub-analysis or we may need to omit this aspect of the analysis, depending on the data that can be extracted from studies.

4.5.2 Approach to Literature Search and Study Selection

The results from the scoping search outlined for research question 1 will also provide the evidence base for research question 3. Citation management and screening practices will also be analogous. The 2013 scoping search identified more than 200 RCTs related to aspects of infection prevention and treatment which may potentially be eligible for this work (**Appendix 1**). Further in-depth screening of these trials will be performed to establish the extent of available data from randomized trials. Following this step, if additional focused searching for non-randomized study data is considered important based on discussion with clinical experts, this will be explored.

4.5.3 Data Collection and Risk of Bias Appraisal

Primary data collection of included studies will be performed independently by two reviewers using a standardized electronic data collection form in DSR. Collected data will be compared for accuracy and agreement, with disagreements being settled by discussion. The following elements will be collected for each included study: study characteristics (authors, year of publication, journal, countries/geographic regions of study performance, and design aspects), patient characteristics (eligibility criteria, number per group, transplant type, and key demographics including age, gender, race, comorbidities, background treatments received, HLA and CMV matching), interventions (drug(s) used as well as dosage and other aspects of administration noted within each study), and outcomes (as mentioned earlier, with number of events and number of patients randomized for binary endpoints, and means with standard deviations for continuous endpoints). As for research questions 1 and 2, the Cochrane Risk of Bias scale will be used to appraise the risk of bias of included studies.

4.5.4 Review of Study Characteristics to Judge Appropriateness of Homogeneity/Similarity Assumption

The importance and approach of establishing homogeneity and similarity when pursuing meta-analyses and network meta-analyses was described in the methods for research question 1. An analogous approach will be employed for research question 3 to compare therapies for prophylaxis/treatment of the types of infections outlined above, with the following patient demographics being carefully reviewed:

- Distribution of disease types amongst patients;
- Distribution of the intensity of conditioning regimens received amongst patients (myeloablative, non-myeloablative, or reduced intensity as described in research question 1);
- Proportion of patients with acute or chronic GVHD;
- Proportion of patients with mismatched HLA status;
- % of patients with a mismatched donor;
- Geographic setting of the study sites (and relation to epidemiologic anti-microbial susceptibility patterns);
- Distribution of fundamental patient demographics (age, comorbidities).

4.5.5 Considerations for Evidence Synthesis

While our literature search will not be limited with regard to interventions sought, the analyses we will undertake in this review will be limited to interventions where at least two eligible RCTs with a minimum of 25 patients per group are available. In setting this criterion, our objective is to generate a more reasonably sized and clinically relevant network of treatments for comparison, as the inclusion of many comparisons that are informed by single studies can be problematic from an analytic perspective and may unnecessarily include interventions of little to no clinical interest. In addition, prior to data extraction, we will present to our clinical experts the list of interventions compared within potentially eligible trials, in order to exclude studies evaluating interventions that are no longer clinically relevant. All studies that are removed for either of the above rationale will be documented in an appendix of the final review.

4.5.5.1 Approach to Synthesis

We will first conduct traditional meta-analyses of all pairwise comparisons in the network to establish the available direct evidence and the corresponding degree of statistical heterogeneity. Network meta-analyses will subsequently be performed.

4.5.5.2 Synthesis of Available Direct Evidence

Standard pairwise meta-analyses will be conducted using random-effects models in Comprehensive Meta-Analyst software to generate summary estimates and to assess statistical heterogeneity. All summary estimates will be reported as odds ratios for binary outcomes and mean differences for continuous outcomes, with corresponding 95% confidence intervals. All measures of I^2 will be reported as proportions ranging from 0–100%.

4.5.5.3 Synthesis of All Available Evidence (Network Meta-Analysis)

A general description of the underlying concepts and applications of network meta-analysis was provided earlier for Research Question 1. Additional considerations of the approach to the network meta-analysis are as follows:

- **Addressing clinical & methodologic heterogeneity**: Primary analyses will be unadjusted; however, additional analyses to assess heterogeneity will be pursued. We will consider additional analyses to address study deficiencies found in risk-of-bias assessments by using meta-regression (depending on the number of included studies) and/or exclusion of low quality studies. Sensitivity analyses using meta-regression and/or reduction of the treatment network to address clinically important variations between studies with regard to gender distribution, age distribution, geographic location of the included studies (considering those in Canada and the US only versus in other locations) and other relevant factors will also be pursued. Tables and figures will be used to show the extent of deviations observed from these analyses in comparison to primary analyses carried out.
- **Clinical subgroup analyses**: specific clinical subgroups of interest in this work will include those defined by site of infection (e.g. respiratory ract, GI, GU, catheter-associated infections, skin and soft tissue, central nervous system, bone and joint).
- **Reporting of findings from analysis**: full graphical and numeric presentations of findings along with a lay-person's summary will be provided. This will include the following: network diagrams showing the availability of evidence for all possible treatment comparisons; summary odds ratios (or mean differences as appropriate), with 95% credible intervals for all pairwise comparisons between regimens; SUCRA and median treatment rankings (with corresponding 95% CrI) for each outcome. These will be described using approaches recommended by Salanti et al.[39] We will use the checklist of the extension of the PRISMA Statement for Network Meta-Analysis to ensure all findings are clearly reported.[40]

- **Assessment of consistency of direct and indirect evidence**: This review will consist of a mixture of both direct evidence and indirect evidence. As described by Dias et al[41] and by Salanti et al[29] in separate publications, there is a need to verify that the findings generated by direct and indirect data do not differ more than one might expect by chance, such that the validity of combining both types of data is questionable. We will employ statistical methods described by Dias et al. to assess the validity of this assumption for all analyses in this review. As statistics alone cannot always be relied upon to identify important clinical or methodologic differences between studies, we will also employ evidence tables of study characteristics to further assess homogeneity. This step will also be important when gathering studies and their data from existing reviews to ensure that all data are relevant to this project.

All network meta-analyses will be performed using WinBUGS software version 1.4.3 (MRC Biostatistics Unit), along with the Microsoft Excel plug-in tool NetMetaXL[42] (for binary and continuous outcomes) to organize all data sets and generate all summary figures including network diagrams, forest plots, league tables and rankograms. Both fixed and random effects models will be fit for each outcome. The fit of a model will be assessed by comparing its posterior residual deviance with the number of unconstrained data points (i.e. the number of intervention arms across all studies) for the analysis. Selection between different models will be based upon deviance information criteria (DIC) for each competing model, with a difference of 5 or more points to be considered significant. Totals of 50,000 or more burn-in iterations and 50,000 or more sampling iterations will be used for all network meta-analyses, and model convergence will be assessed based on inspection of history plots and the Monte Carlo error of all parameters.

4.6 Perceived Challenges of this Review

The extent of available data related to preventive and treatment strategies from randomized trials and the design features of these trials is unclear and will be closely studied during screening. The extent to which different types of transplants involved (i.e. allogeneic, autologous data; and potential mixture with other hospitalized patients), along with other features, will be discussed with clinical experts as the review progresses. If some restrictions with regard to population must be relaxed to incorporate the relevant information, this will be determined with our participating clinical experts. As mentioned earlier, additional searching will be considered if the evidence base from randomized trials is found to be limited.

4.7 Knowledge Translation Plans

In working with the CBMTG and pCODR on this review, we intend to provide appropriately detailed reports to both organizations to inform future clinical guidance and/or changes in practice that may subsequently be pursued. Sub-committees of the CBMTG will use the data from our analysis to formulate best practices and inform member transplant centres of recommendations and guidelines. Moreover, the clinical trials network of the CBMTG will use the information to plan and execute future studies to generate new knowledge and improve care for patients undergoing allogeneic HSCT. Cancer Care Ontario's Stem Cell Transplant Steering Committee will be engaged to disseminate findings within the Provincial transplant programs and identify opportunities for future program development. We will also present this information at meetings to both groups as needed for dissemination of findings. Key representatives from our knowledge users will also participate throughout our review process as suggested for an integrated KT approach; they will have the opportunity to provide input at various key landmarks during the review process, including review of study characteristics and discussion of preliminary findings from our analyses. We will also share the findings from our work with DSEN once the review is complete. One or more peer-reviewed publications will be pursued, dependent upon the volume of final results that are generated from our work; we will also publish the protocol and register it with the PROSPERO database of systematic reviews. Submissions for oral presentations at relevant conferences and to relevant stakeholders will be planned.

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Appendix 1: Literature Search Strategy for Scoping Review

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present> **June 12, 2013**

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1 Hematopoietic Stem Cell Transplantation/
2 ((h?ematopoietic adj3 transplant$) or hsct$).tw.
3 peripheral blood cell transplant$.tw.
4 peripheral blood stem cell transplant$.tw.
5 Bone Marrow Transplantation/
6 bone marrow transplant$.tw.
7 STEM CELL TRANSPLANTATION/
8 stem cell transplant$.tw.
9 stem cell therap$.tw.
10 PERIPHERAL BLOOD STEM CELL TRANSPLANTATION/
11 peripheral stem cell transplant$.tw.
12 H?ematopoietic peripheral blood stem cell transplant$.tw.
13 or/1-12
14 randomized controlled trial.pt.
15 controlled clinical trial.pt.
16 randomized.ab.
17 placebo.ab.
18 clinical trials as topic.sh.
19 randomly.ab.
20 trial.ti.
21 or/14-20
22 exp animals/ not humans.sh.
23 21 not 22
24 13 and 23

Results from Screening of Abstracts and Full texts