A phase I / II study of pegylated-Interferon-2alpha for relapsed haematological malignancy after allogeneic haematopoietic progenitor cell transplantation (HPCT)

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SYNOPSIS

***Background*:** Allogeneic haematopoietic progenitor cell transplantation (HPCT) is the only available potentially curative therapy for a range of malignant conditions, particularly acute leukaemia. The curative potential of HPCT relates to the graft-versus-leukaemia effect whereby residual recipient leukaemia is immunologically eliminated by NK and T cells within the transplanted donor immune system. Patients in whom the GVL effect has failed and relapse with leukaemia after HPCT have a very poor prognosis and survival at two years of <10%. The prognosis is worst for patients relapsing early after transplant or those that relapse despite significant GVHD. Clearly new therapies are urgently needed for this group of very poor prognosis patients. Type I interferons (e.g. IFNα) are critical cytokines controlling the GVHD and GVL response after HPCT. Preclinical data suggests that the administration of these cytokines after HPCT can significantly improve GVL by sensitizing the residual malignancy to immunologically mediated cytotoxicity whilst concurrently increasing the magnitude of the donor cytotoxic response. New pegylated versions of IFNα are now available that have biologically relevant half lives and thus enhanced activity. These agents represent an attractive biological modifier to improve GVL responses after HPCT.

***Aims*:** To determine the safety and efficacy of FLAG reinduction followed by pegylated-IFN-2α with or without Donor Lymphocyte Infusions (DLI) to prolong survival in patients in relapse after allogeneic HPCT.

***Study Design*:** Phase I / II study design.

***Population*:** Eligible patients are those that have relapsed disease after initial sibling or volunteer unrelated allogeneic HPCT.

***Treatment*:** (*Cohort A*) Patients in haematological relapse will be reinduced with FLAG (for acute leukaemia) or NHL-targeted chemotherapy without ongoing pharmacological GVHD prophylaxis. Supportive care, including antibiotic prophylaxis and nutritional support will also be administered as per standard Institutional guidelines. Peg-IFN (Pegasys®) will commence at day 21 after chemotherapy or at count recovery (whichever is later) in the absence of significant acute GVHD (grade II or higher) at a dose of 45mcg weekly subcutaneously, increasing to 90mcg, 135mcg and then 180mcg each week as tolerated and then to continue at 180mcg weekly for a total of 6 months. In the absence of grade II-IV acute GVHD or progressive chronic GVHD, DLI may commence at day 35. (*Cohort B*) Patients with non-haematological relapse (detectable only by cytogeneic, molecular or flow cytometry based assays) may receive peg-IFN-2α (Pegasys®) without FLAG reinduction. Patients in non-haematological relapse with adequate marrow function can receive peg-IFN immediately if off immune suppression without significant acute GVHD (grade II-IV) or progressive chronic GVHD and proceed to DLI at day 35 if progressive disease is evident.

***Outcomes*:** The primary endpoint is overall survival (OS) at 2 years. Secondary endpoints include the incidence of acute GVHD (grade II-IV), incidence and severity of toxicity (cytopenia, constitutional symptoms, depression) and relapse.

***Assessment*:** GVHD will be staged and graded according to standard Seattle criteria. GVHD assessment, assessment of infective complications, toxicity, relapse and OS will occur at 1, 2, 3, 6, 9, 12 and 24 months. Cytokine and cellular responses will be measured regularly up until two years.

***Statistics*:** Analysis of a historical HPCT patient cohort at RBWH receiving FLAG chemotherapy demonstrates that overall survival at 2 years in this cohort is 7%. To detect an

improvement to 40%, with 80% power and a two-sided significance of 5%, a sample size of 29 patients is required for scheme A. The scheme B cohort will be a smaller group, the study is thus not powered by this cohort and will be analysed separately.

***Feasibility*:** The trial will be conducted locally with an estimated 10 patients recruited per year in scheme A. Enrolment is expected to be completed within 36 months.

OBJECTIVES

This study aims to assess the safety and efficacy of pegylated-interferon-2alpha with or without donor lymphocyte infusions for patients that have relapsed acute leukaemia after allogeneic haematopoietic progenitor cell transplantation (HPCT).

1. BACKGROUND

1.1 *Relapsed disease after HPCT*

Allogeneic HPCT is the only currently available potential curative therapy for a range of malignant and non-malignant conditions. The curative potential of allogeneic HPCT in treatment of malignancy is largely mediated through allogeneic immune responses directed towards residual cancer cells present at transplantation. This type of immune response is termed a “graft versus Leukaemia” (GVL) effect.

Unfortunately, allogeneic immune responses in HPCT can also be directed towards normal host (patient) organs and tissues, potentially resulting in significant morbidity and / or mortality. This type of immune response is termed (acute) graft versus host disease (GVHD). Differentiating GVHD from GVL immune responses enables the potential to limit post-transplant morbidity / mortality whilst maintaining the therapeutic potential of HPCT. Despite allogeneic HPCT being the most potent anti-leukaemic modality currently available, up to 40% of patients still relapse and all these patients will die without further intervention. Patients in whom the combined therapeutic modalities of chemoradiotherapy the GVL effects with allogeneic HPCT have failed and relapse have a very poor prognosis with long term survival of less than 10% at RBWH. This data is shown below:

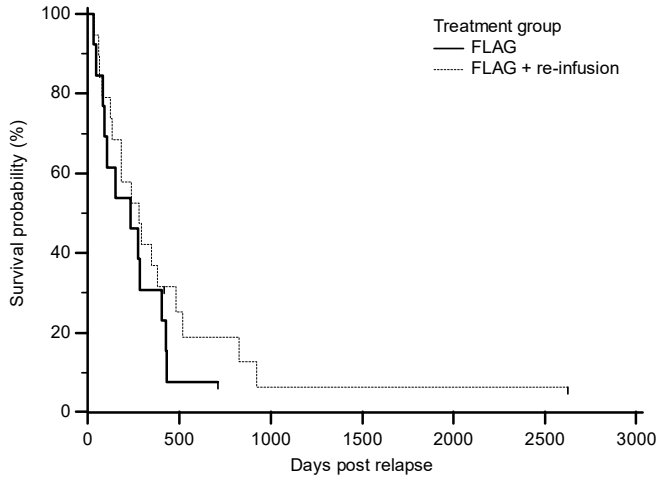


Figure 1: Survival in patients who relapse after allogeneic HPCT at RBWH. Patients are divided into those that received FLAG based re-induction only (n = 13) or FLAG based re-induction and a second allograft from the same donor (n = 19). Neither group received immune suppression at the time of FLAG chemotherapy. Survival in the two arms is not different (*P* = 0.34) and mortality reflects leukaemia-related death.

There are thus no standard therapeutic approaches in patients who have relapsed after allogeneic HPCT and outcomes are dismal. Some patients relapsing more than 12 months after HPCT can be salvaged with induction chemotherapy and/or donor lymphocyte infusions/second stem cell grafts but in general the outcome remains poor. Overall survival beyond two years is less than 20% and approaches 0% for patients relapsing within 6 months of HPCT.[1-5]

1.2 *GVHD and GVL Biology*

Acute GVHD is generally defined as occurring within three broad stages:[6]

1. Conditioning invokes recipient tissue damage and subsequent inflammation.
2. Donor T cells are primed by recipient Antigen Presenting Cells (APC), and differentiate within a Th1 paradigm.
3. Target tissue apoptosis is mediated by cytolytic cellular and cytokine effectors.

Acute GVHD occurs early in the transplant period and is absolutely dependent on the presence and function of donor T cells in the donor inoculum.[7-9] Following HPCT, tissue injury and inflammation characterized by proinflammatory cytokine release (TNF and IL-1) is initiated by the conditioning regimen. These cytokines, together with lipopolysaccharide (LPS) released from damaged gut tissue, results in the activation of host APC. Activated host APC then prime naïve donor T cells and preferentially drive Th1 differentiation and expand effector $CD8^+$ T cells which mediate target tissue GVHD in the cytolytic effector pathway. In concert, LPS triggers the release of cytopathic quantities of inflammatory cytokines from monocytes and macrophages, generating the classical "cytokine storm". Thus, acute GVHD can be defined as a Th1 paradigm which results in extensive tissue destruction characterized by apoptosis.

Therapeutic GVL responses are mediated by donor NK cells and T cells and the antigenic targets are principally minor histocompatibility antigens (mHA), although hematopoietic-specific responses also occur. Recipient mHA are predominantly presented directly to donor $CD8^+$ T cells by recipient hematopoietic-derived APC within the first few weeks following transplantation, prior to their replacement by donor-derived APC[10]. These donor-derived APC must subsequently cross-present recipient mHA to donor $CD8^+$ T cells. Both $CD4^+$ and $CD8^+$ T cells are important in GVL responses, with $CD8^+$ T cells mediating direct cytotoxicity against leukemic cells while $CD4^+$ T cells are capable of both direct cytotoxicity (if the leukaemia is MHC class II positive) and providing helper function to donor $CD8^+$ T cells (via cytokines such as IL-2 and the licensing of APC). Following recognition of target antigen on leukaemia cells, cytotoxic effector populations can mediate damage via multiple different pathways including secretion of perforin and granzymes or through Fas-FasL, TNF and/or TRAIL[11,12]. While T cells deficient in both Fas ligand and perforin have been demonstrated to exert GVL activity *in vivo*[13], these molecules are clearly required for meaningful GVL activity[14,15].

The generation of GVHD and an effective GVL response is thus a complex process requiring the interaction of an APC presenting relevant mHA with a donor T cell and subsequent differentiation and acquisition of effector function. As described below, the IFNs are key molecules in orchestrating this immune response.

1.3 *The Interferons*

The interferons were discovered by Issaacs and Lindenmann in 1957 when they were investigating viral interference and they have subsequently been defined by this ability[16]. It was not until 1978 that they were able to be purified, analyzed and characterized[17]. They are recognized as a key component of innate immunity and the first line of defence against viral infection. There are three distinct types (type I, type II and type III), which are distinguished based on their structure, cognate receptors and biological activities. The type I IFNs are well characterized and are the hallmark interferon important in defence against viral infection. In contrast, the type II IFN's are better known for mediating immune responses to a broad range of intracellular pathogens. The type III IFN's are more recently described and not well characterized, however they have similar downstream effects as type I IFN's. Therefore, while

IFN's were initially described due to their antiviral properties, they exhibit multiple distinct additional immunological properties.

Type I Interferons

The type I IFNs encompass a large family of cytokines that include a single IFN-β isotype, greater than 13 IFN-α isotypes and multiple other less described subtypes[18]. Most cell types can be induced to secrete Type I IFN in response to viral infection. The major pathway through which this occurs is activation of the cytosolic receptors retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (Mda5) by double stranded RNA[19]. Type I IFN production is induced following activation of five of the eleven Toll like receptors (TLR)[20]. However, the most potent producers are plasmacytoid DC (pDC)[21], which preferentially express TLR7 and TLR9 that recognize single stranded RNA and CpG-rich DNA respectively[22]. Following engagement of these receptors, the MyD88-IFN regulatory protein (IRF7) pathway is crucial for type I IFN production, with extended retainment of CpG and MyD88/IRF7 complexes in endosomal vesicles responsible for the ability of pDC to induce high levels of Type I IFN[23]. All Type I IFN signal through the same receptor, which is composed of 2 subunits IFNAR1 and IFNAR2[24]. Importantly, the mouse has a comparable type I IFN system to human, with multiple cytokine subsets and both IFNAR1 and IFNAR2 components of the receptor[24], which is expressed on essentially all cells[25]. Following signaling through the type I IFN receptor, downstream responses are transmitted through the kinases tyrosine kinase 2 and JAK1, which recruits STAT1 to form the STAT1-STAT2 heterodimer complex that dissociates and migrates into the nucleus[18]. In the nucleus, this heterodimer associates with interferon regulatory factor 9 to form the IFN-stimulated gene factor 3 that binds to the IFN-stimulated response element promoter sequence to activate transcription of the IFN inducible genes[18].

Type I IFN signaling is important in both the innate and adaptive immune responses. These effects were initially studied in the context of viral infection, with signaling resulting in anti-proliferative responses in conjunction with up-regulation of MHC class I expression[26]. During the adaptive immune response type I IFN signaling is vital for the expansion and differentiation of effector CTL's[27]. The injection of synthetic dsRNA (poly(I:C)) to induce Type I IFN augments the response of TCR transgenic $CD8^+$ T cells to directly presented antigen. Increased levels of Type I IFN have also been shown to promote cross presentation of exogenous recipient mHA to $CD8^+$ T cells by APC, a process which is independent of $CD4^+$ T cell help [28]. Bystander activation of T cells can also occur in the presence of high levels of Type I IFN, as proliferation is observed without up-regulation of CD69 and CD25[29]. More recently there has been renewed interest in the role of type I IFN in immunoediting and tumor clearance. Early NK anti-tumor responses are dependent on Type I IFN signaling[30]. Homeostatic numbers of NK cells in mice with a deficiency of both the AR1 and AR2 components of the IFNAR are reduced but the cytotoxicity activity of naive NK cells is similar[30]. However, IL-2 mediated activation and subsequent killing is defective in mice lacking the Type I IFN receptor[30]. This suggests that while Type I IFN signaling does not affect the baseline activity of NK cells, it serves to enhance

anti-tumor responses following activation. Type I IFN signaling is also important for CD8 T cell responses against tumor. The induction of Type I IFN with CpG via the TLR9 receptor in combination with DC-targeted vaccines effectively activates tumor-specific $CD8^+$ T cells leading to prolonged survival in tumor models, and increased serum levels of IFN-α are observed [31]. Further evidence for the importance of Type I IFN in anti-tumor function demonstrates that they play a role in immunoediting[32]. IFNAR1 deficient mice are more susceptible to induced sarcomas and tumors arising in these mice have an 'uneditited' phenotype, as they are rapidly rejected via a T cell dependent mechanism when transplanted into wild-type mice.[32] Interestingly, tumor rejection occurs via effects on hematopoietic cells, rather than via direct effects on tumor cells[32]. Therefore type I IFN contributes to both innate and adaptive arms of the anti-tumor immune response.

In conjunction with effects observed through NK and CD8 T cells, type I IFNs also modulate DC function. Type I IFN promotes the maturation of DC[33] and enhances cross-presentation to CD8 T cells by $CD8\alpha^+$ DC and subsequent rejection of tumor[34,35]. The mechanism by which IFN-β administration induces beneficial outcomes in multiple sclerosis patients has been investigated in experimental autoimmune encephalitis[36]. These studies demonstrate that type I IFN signaling down-regulates the Th17 mediated inflammatory response by inhibiting osteopontin expression in DC, resulting in derepression of the potent inhibitory cytokine IL-27[37,38]. The ability of type I IFN signaling to reduce IL-17 production has also been documented in patients with ulcerative colitis, with treatment over a period of 12 weeks resulting in a significant decrease in IL-17A mRNA expression in colonic biopsies.[39] Therefore type I IFN signaling in DC modulates subsequent cellular and cytokine differentiation responses.

1.4 *Role of Type I IFN in GVHD*

1.4.1 *Clinical data*

IFN-α was routinely used to treat patients with chronic myeloid leukemia in the pre-tyrosine kinase (i.e. imatinib, dasatinib, nilotinab) era, with responses thought primarily to relate to anti-proliferative effects[40,41].

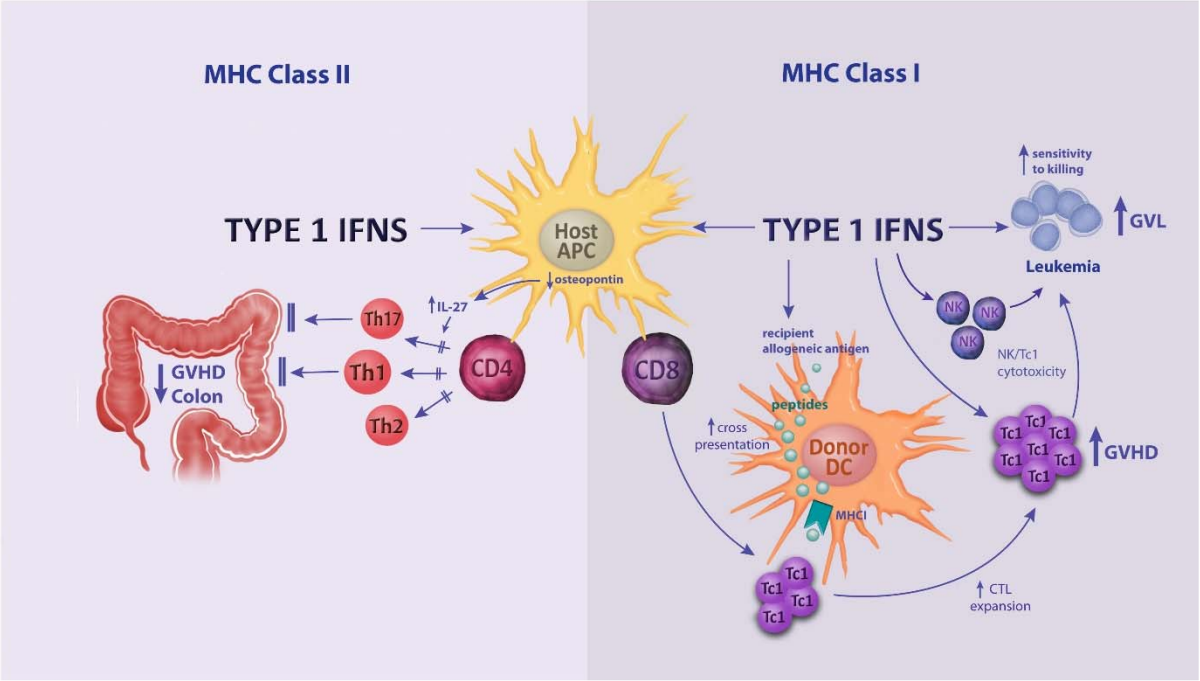


Figure 2. The differential effects of Type I IFN signaling on MHC Class I and MHC Class II dependent GVHD and GVL responses. Signaling on host hematopoietic tissue decreases donor CD4 T cell proliferation and differentiation via inhibitory effects on recipient APC, resulting in reduced T cell differentiation and GVHD of the colon. The inhibition of Th17 differentiation has been associated with inhibition of APC-derived osteopontin which promotes IL-27 secretion. The signaling of donor APC by Type I IFN promotes cross-presentation (i.e. the presentation of exogenous recipient allogeneic peptides within MHC class I) and the expansion of donor CTL. This effect is further amplified by direct signaling to donor NK and CD8 T cells which together promotes cytotoxicity (e.g. mediated by perforin/granzyme) against GVHD target tissue and residual recipient leukemia (i.e. GVL). Finally, the type I IFNs enhance the susceptibility of residual leukemia to CD8 and NK cell mediated cytotoxicity, further amplifying GVL responses.

IFN-α administration is now undergoing a renaissance as combination therapy with tyrosine kinase inhibitors in an effort to promote leukemia stem cell cycling and render these cells sensitive to the latter agents. The finding that IFN-α administration *in vivo* induces the transition of HSC from a dormant stage into active cell cycle was surprising, considering the anti-proliferative activity observed in *in vitro* culture systems[42]. The increased proliferation of HSC results in increased susceptibility to elimination by anti-proliferative chemotherapeutic drugs. Clinical studies investigating the effects of IFN-α administration after allogeneic SCT demonstrated enhancement of both GVHD and GVL responses[43-45]. The effects of IFN-α and mechanism of disease control was recently addressed by our group in preclinical models. This study demonstrated differential effects on donor $CD4^+$ and $CD8^+$ T cells[46]. Type I IFN signaling indirectly inhibits donor $CD4^+$ T cells, via recipient antigen presenting cells, resulting in alleviation of GVHD (see Figure 2). In contrast, signaling directly enhanced donor $CD8^+$ T cell responses and increased susceptibility of host cells to killing by donor $CD8^+$ T cells, both promoting GVHD and improving protective GVL responses. A mild reduction in hepatic GVHD histopathology in the absence of type I IFN signaling has also been demonstrated in a system of GVHD directed towards miHA only[47]. Clinical data from the 1990's demonstrated a detrimental effect of type I IFN administration prior to transplant, with increases in severe GVHD[48,49]. This is consistent with MHC class I restricted effects on GVHD in clinical

transplantation where donor and recipients are HLA-matched. It was also recently demonstrated that type I IFN enhances the cross-presentation of exogenous recipient antigens by donor APC within MHC class I after BMT[50]. The contribution of cross-presentation to GVL responses was not investigated in our studies due to the models utilized being MHC mismatched, but this effect would be expected to enhance therapeutic GVL responses in patients.

While this data suggests there would be improved tumor clearance following treatment with IFN-α prior to transplant, clinical SCT data in the CML setting combined with our own data suggest that the detrimental effects of GVHD overwhelm this protective response. Therefore since extensive data indicate that type I IFN signaling after SCT promotes GVL responses (as outlined above), the most effective therapeutic use of type I IFN is likely in SCT patients at a high risk of relapse. In this setting type I IFN would be predicted to increase the sensitivity of malignancy to killing whilst enhancing the donor NK and $CD8^+$ T cell responses that mediate GVL. Whether newer pegylated versions of type I IFN will be more efficacious in this setting is a critical issue.

1.5 *Pegylated IFN*

Pegasys® is a recombinant interferon-2alpha protein produced by recombinant DNA technology within E coli and then conjugated to a polyethylene glycol chain (PEG) molecule of 40 kilodaltons. Pegylation of the native IFN molecule eliminates renal excretion and prolongs the drug half-live from 3-4 hours to 160 hours after subcutaneous dosing, reaching steady state in 5-8 weeks. For this reason pegylation of IFN results in prolonged action and avoids the need for the daily dosing required for the native cytokine and the frequent peak and troughs seen therein. In general pegylation of cytokines result in significantly prolonged activity and enhanced potency *in vivo*.[9,51]

Importantly, the administration of pegylated-IFN after allogeneic BMT has not been studied previously.

2. STUDY RATIONALE

There are no standard approaches to treat haematological malignancies that relapse after allogeneic HPCT. To date, the outcome of retrospective and prospective phase I/II studies demonstrate that in this setting:

1) Donor lymphocyte infusions can achieve long term disease control in between 10 and 20% in non-CML patient groups relapsing late after HPCT.[1,2]
2) Debulking of disease with induction chemotherapy can result in short-term disease control.[2,5]
3) The induction of lymphopenia by chemotherapy (e.g. with FLAG or Flu/Cy) can revert T cells tolerant to nominal antigens to a functional status[52] and improve the efficacy of GVL.[53]
4) That type I interferons promote potent GVL effects in both preclinical[46] and anecdotal clinical studies.[54]

5) Pegylation of native cytokines dramatically improves their biological half-life and functional efficacy in vivo.[9,55]

We therefore propose a step-wise approach to management of relapsed malignancy post HPCT incorporating pegylated-IFNα within the treatment algorithm. Patients will initially have their immunosuppression withdrawn, and in the presence of frank haematological relapse, also undertake FLAG induction chemotherapy as a platform to provide both short-term disease control as well as lymphodepletion. In the absence of development of subsequent GVHD, escalating doses of pegylated-IFNα (Pegasys®) will be commenced. If after achieving maximal doses of pegylated-IFNα (180mcg/week) significant GVHD has not developed, depending on donor availability, patients will also be eligible to commence donor lymphocyte infusions (DLI) whilst continuing pegylated-IFNα.

This approach will (i) permit the use of chemotherapy without the induction of severe GVHD that is seen in the majority of patients that receive a second stem cell graft[54] (ii) whilst permitting peg-IFN to be delivered to the majority of recipients and (iii) allowing DLI to be administered thereafter to poor responders.

3. PATIENT SELECTION

3.1 *Inclusion Criteria*

3.1.1 Patients with relapse of their primary disease after allogeneic HPCT (either sibling or VUD donors).

3.1.2 For cohort A, have received further chemotherapy as defined in treatment plan (pg 11)

3.1.3 For cohort B, are NOT in haematological relapse and have evidence of disease ONLY by flow cytometry, cytogenetics or molecular assays.

3.1.4 Resolution of cytopenias prior to commencing peg-IFN as defined in section 4.

3.1.5 Age ≥18 and <65 years

3.1.6 Eastern Cooperative Oncology Group (ECOG) performance status ≤3 (Karnofsky ≥50%; see Appendix A**)**

3.1.7 Off immune suppression

- Patients are allowed to continue on prednisolone (or equivalent) at doses <0.5mg/kg/day.
- Patients must have ceased Cyclosporine (CSA), Tacrolimus (Tacro), Mycophenolate Mofetil (MMF) and / or all other immunosuppressants.

3.1.8 Absence of active significant GVHD off immunosuprression (as per Section 3.1.4), as defined by <grade II acute GVHD and / or progressive or extensive stage chronic GVHD.

3.1.9 Adequate organ function for FLAG or alternate lymphoma directed chemotherapy (if in haematologic relapse) as per Institutional guidelines, defined in Table 1 below**:**

Table 1

Total bilirubin	≤30µmol/L
Creatinine clearance	≥50 mL/min/1.73 m^2 for patients with creatinine levels above ULN

ULN = upper limit of normal at local institutional laboratory

Able and willing to provide written informed consent

3.2 *Exclusion Criteria*

3.2.1 Inadequate organ function for FLAG chemotherapy (if in haematological relapse) as per Institutional guidelines, defined in Table 2 below**:**

Table 2

Total bilirubin	>30µmol/L
Creatinine clearance	<50 mL/min/1.73 m^2 for patients with creatinine levels above ULN

3.2.2 Haematological relapse if enrolling in cohort B.

3.2.3 Active acute (grade II-IV) or progressive and / or extensive stage chronic GVHD requiring immune suppression with prednisone (or equivalent) at doses ≥0.5mg/kg/day or ongoing therapy with other immunosuppressant medications including calcineurin inhibitors (cyclosporine, Tacrolimus) and MMF.

3.2.4 Patients receiving any other investigational agents**.**

3.2.5 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, unstable angina pectoris, uncontrolled hypertension or heart failure, uncontrolled diabetes, uncontrolled autoimmune disease (especially thyroid), uncontrolled COPD, uncontrolled depression, epilepsy and social situations that would limit compliance with study requirements**.**

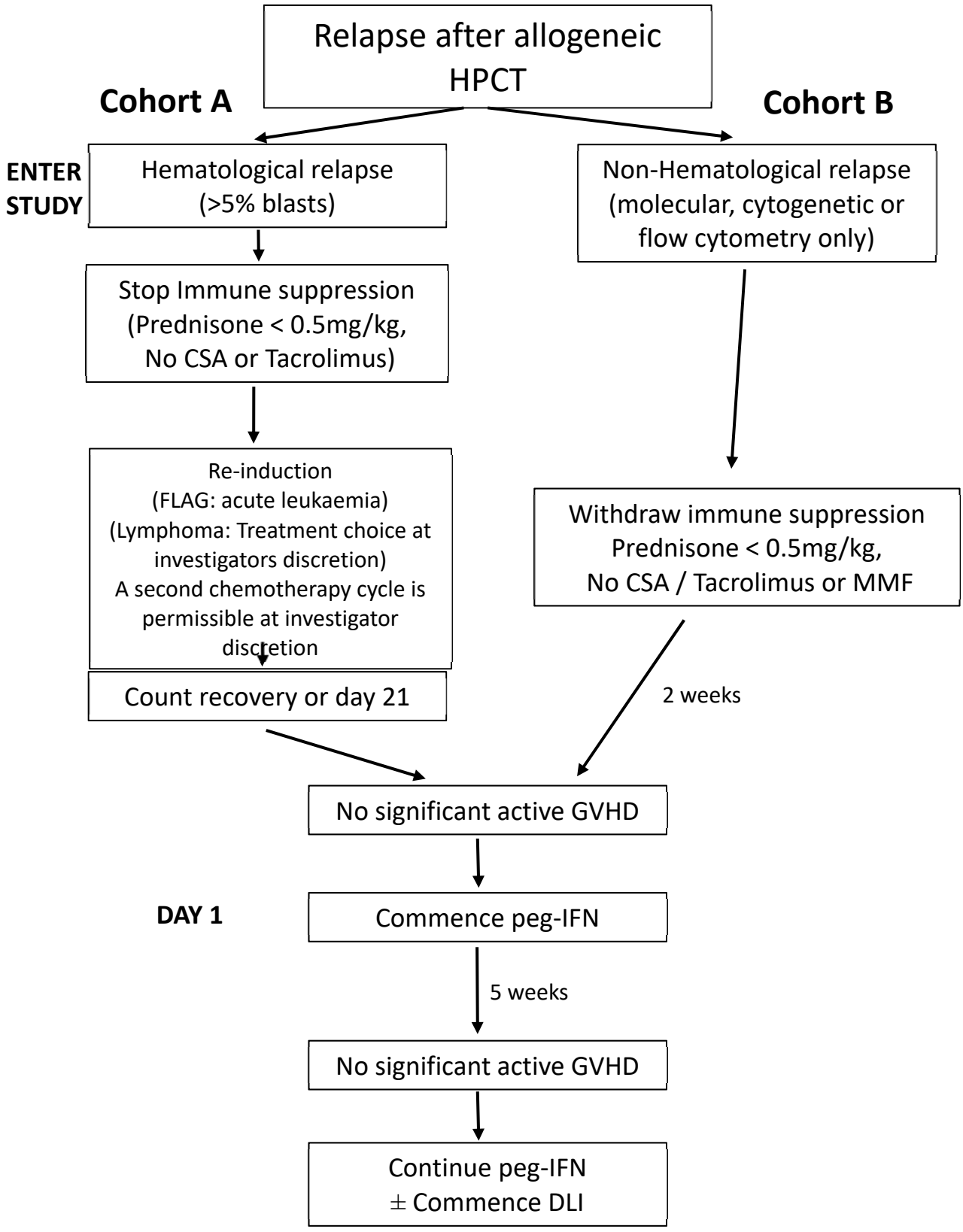
3.2.6 Known HIV infection.

3.2.7 Pregnant or breastfeeding, or patient with reproductive potential who is not willing to use adequate contraceptive precautions in the judgement of the Investigator. Adequate contraception is defined as a double-barrier method, i.e. using at least 2 methods of contraception e.g. 2 actual barrier methods or 1 actual barrier method and 1 hormonal method.

3.2.8 Donor is an identical twin (i.e. syngeneic)

3.2.9 History of allergic or grade IV reactions to interferon, including known allergies to E coli-derived products eg. G-CSF.

4. TREATMENT PLAN (see algorithm)



4.1 *Study entry*

Patients will enter the study at diagnosis of relapsed malignancy post HPCT.

Depending on the bulk of malignancy at relapse, patients will either undertake re-induction chemotherapy with FLAG (Cohort A), or simply undertake withdrawal of immunosuppression alone (Cohort B) prior to commencing peg-IFN.

4.1.1 *Cohort A*

Patients with frank haematological relapse will be treated along Cohort A. "Haematological" or "frank" relapse is defined by ≥5% blasts (or the malignant clone in case of myeloma, lymphoproliferative of myeloproliferative disorders) in bone marrow or blood in leukemia patients, or pathologically enlarged lymphadenopathy (≥1cm) on either clinical or CT examination in patients with lymphoproliferative disorders.

Patients entering the cohort A pathway will have their immunosuppression ceased either prior to or at commencement of re-induction. This includes cessation of Cyclosporine (CSA), Tacrolimus (Tacro), Mycophenolate Mofetil (MMF) and / or all other immunosuppressants, with exception of prednisolone (or equivalent), which may be continued at doses <0.5mg/kg/day.

For patients with acute leukaemia, FLAG will be administered on an inpatient basis, as per institutional guidelines. The schedule is:

- Fludarabine 30mg/m^2 IV daily (over 30 minutes) on days 1-5 *followed 4 hrs later by*
- Cytarabine 2000mg/m^2 IV daily (over 4 hours) on days 1-5 *followed by*
- G-CSF (5ug/kg) SC daily from day 6 to neutrophil recovery

A second induction cycle of the investigators choosing may be given prior to commencing peg-IFN.

For patients with lymphoma, up to two cycles of chemotherapy may be delivered, chosen at the investigators discretion, prior to commencing peg-IFN. The commencement of peg-IFN will remain as per section 4.2.

4.1.2 *Cohort B*

Patients without "haematological" or "frank" relapse of their underlying malignancy (as defined in 4.1.1) will simply have their immunosuppression withdrawn prior to commencement of peg-IFN. Patients in cohort B must be off their immunosuppression, as defined by cessation of Cyclosporine (CSA), Tacrolimus (Tacro), Mycophenolate Mofetil (MMF) and / or all other immunosuppressants, for at least 2 weeks before commencing peg-IFN. Of

note, patients may continue on steroids at prednisolone (or equivalent) doses of <0.5mg/kg/day during this time.

4.2 *Commencement of Peg-IFN*

4.2.1 Cohort A

In the absence of development of ≥grade II GVHD and / or progressive or extensive stage chronic GVHD, patients will commence Peg-interferon (Pegasys®) either at day 21 post chemotherapy and at a time of adequate marrow function.

Adequate marrow function is defined as an ANC > 1 x 10^9/L and plts > 50x10^9/L (independent of G-CSF or platelet transfusions for 5 days).

If cytopenias relate to persistent disease adequate marrow function will be defined as ANC > 0.5 x 10^9/L and plts > 30x10^9/L (independent of G-CSF or platelet transfusions for 5 days). In this scenario thresholds for dose reductions will be as per section 4.3.6.

Patients do not have to be in complete haematological remission to receive peg-IFN after chemotherapy although this may be desirable for non-myeloid malignancies.

Pegasys® will commence at a dose of 45mcg SC once per week, with weekly escalation of dose to 90mcg, then 135mcg then 180mcg if tolerated. Patients will then continue peg-IFN as “maintenance” at 180mcg weekly for a total of 6 months (calculated from first dose of peg-IFN) if able.

4.2.2 *Cohort B*

In the absence of development of ≥grade II GVHD and / or progressive or extensive stage chronic GVHD patients will commence Peg-interferon (Pegasys®) at 14 days post withdrawal of immunosuppression if marrow function is adequate.

Adequate marrow function is defined as an ANC > 1 x 10^9/L and plts > 50x10^9/L (independent of G-CSF or platelet transfusions for 5 days).

Pegasys® will commence at a dose of 45mcg SC once per week, with weekly escalation of dose to 90mcg, then 135mcg then 180mcg if tolerated. Patients will then continue peg-IFN as "maintenance" at 180mcg weekly for a total of 6 months (calculated from first dose of peg-IFN) if able.

4.3 *PEG-IFN dose adjustments*

4.3.1 Cytopenias (see Table below)

Patients will be monitored with at least weekly FBC during the dose escalation phase of peg-IFN, then at least 2nd weekly during the maintenance phase of peg-IFN administration.

If cytopenias are secondary to disease only then peg-IFN dose adjustments are not required if platelets can be supported at > 20 and ANC > 0.5 x 10^9.

For cytopenias suspected to be related to peg-IFN (rather than disease progression) the following peg-IFN dose adjustments are recommended:

If ANC <0.75 x 10^9 **or** platelets <50 x 10^9:

- Reduce peg-IFN dose to the previous dose level (dose levels are 45mcg, 90mcg, 135mcg, 180mcg)
- Remain on reduced dose for a minimum of 14 days
- Only increase dose to next dose level when counts are stable for 14 days ie. if ANC >0.75 x 10^9 and platelets >50 x 10^9.

If ANC <0.5 x 10^9 despite G-CSF or platelets <25 x 10^9:

- STOP peg-IFN
- Restart peg-IFN at two doses lower on count recovery ANC >0.75 x 10^9 and platelets >50 x 10^9
- Only, increase dose to the next dose level when counts are stable for 14 days ie. ANC >0.75 x 10^9 and platelets >50 x 10^9.

Patients WITHOUT frank haematological disease AND falling counts secondary to peg-IFN:

Peg-IFN dose at time of cytopenia	ANC ≥0.5 <0.75 OR Plts ≥25 <50	ANC <0.5 or plts <25	GVHD ≥grade 2 or progressive extensive stage chronic

	New PEG IFN dose	Stop PEG IFN dose until count recovery ###	PEG-IFN dose at recommencement (re-start at x2 dose levels lower)	Stop PEG IFN dose until GVHD quiescent$$$	PEG-IFN dose at recommencement
0	0	0	0	0	0
45mcg	0	0	0	0	0
90mcg	45	0	0	0	45
135mcg	90	0	45	0	45
180mcg	135	0	90	0	45
	Keep on this dose for minimum of 2 weeks***		Keep on this dose for minimum of 2 weeks***		Keep on this dose for minimum of 4 weeks &&&

*** If after 2 weeks at reduced dose, ANC recover to >0.75 and plts ≥50, then PEG-IFN dose increments can recommence BUT now at 2 weekly intervals for all dose increments (not weekly).

Count recovery is defined as ANC >0.75 and plts >50 on x2 sequential weekly FBC readings.

&&&If no new and / or progressive GVHD (≥grade II acute GVHD or progressive chronic GVHD) after 4 weeks after PEG-IFN recommenced, then dose escalation may occur every 4 weeks to a maximum dose one level below that in which GVHD initially commenced

$$$ Restart at peg-IFN at 45mcg only when resolution of acute GVHD (to grade 0/1) or quiescent chronic GVHD irrespective of other immunosuppression

Patients WITH frank haematological disease AND falling counts secondary to peg-IFN:

Peg-IFN dose at time of cytopenia	ANC <0.5 OR Plts ≥20 <30	plts <20		GVHD ≥grade 2 or progressive extensive stage chronic	
	New PEG IFN dose	Stop PEG IFN dose until count recovery ###	PEG-IFN dose at recommencement (re-start at x2 dose levels lower)	Stop PEG IFN dose until GVHD quiescent$$$	PEG-IFN dose at recommencement
0	0	0	0	0	0
45mcg	0	0	0	0	0
90mcg	45	0	0	0	45
135mcg	90	0	45	0	45
180mcg	135	0	90	0	45
	Keep on this dose for minimum of 1 week***		Keep on this dose for minimum of 1 week***		Keep on this dose for minimum of 4 weeks &&&

*** If after one week at reduced dose, ANC recover to ≥0.5 and plts ≥30, then PEG-IFN dose increments can recommence at weekly intervals for all dose increments.

Count recovery is defined as plts >20 on weekly FBC readings.

&&&If no new and / or progressive GVHD (≥grade II acute GVHD or progressive chronic GVHD) after 4 weeks after PEG-IFN recommenced, then dose escalation may occur every 4 weeks to a maximum dose one level below that in which GVHD initially commenced

$$$ Restart at peg-IFN at 45mcg only when resolution of acute GVHD (grade 0/1) or quiescent chronic GVHD irrespective of other immunosuppression

4.3.2 *GVHD*

Progressive GVHD despite systemic steroids (1mg/kg), severe acute GVHD (grade II-IV acute GVHD) or progressive extensive chronic GVHD:

- STOP peg-IFN
- Restart at peg-IFN at 45mcg only when resolution of acute GVHD (grade 1) or quiescent chronic GVHD irrespective of immunosuppression
- Dose escalation may occur every 4 weeks to a maximum dose one level below that in which GVHD commenced.

4.3.3 *Renal impairment*

If CrCl <30ml/min, the maximum dose of peg-IFN is 135mcg/week

4.3.4 Decreased or loss of vision, colitis or pancreatitis

Stop peg-IFN and seek urgent sub-specialist review.

4.3.5 *Depression*

Depression Severity	**Initial Management (4-8 weeks)**		**Depression Status**		
	Dose modification	**Visit schedule**	**Remains stable**	**Improves**	**Worsens**
Mild	No change	Evaluate once weekly by visit and/or phone	Continue weekly visit schedule	Resume normal visit schedule	(See moderate or severe depression)
Moderate	Decrease PEGASYS dose to 135 mcg (in some cases dose reduction to 90 mcg may be needed)	Evaluate once weekly (office visit at least every other week)	Consider psychiatric consultation. Continue reduced dosing	If symptoms improve and are stable for 4 weeks, may resume normal visit schedule. Continue reduced dosing or return to normal dose	(See severe depression)
Severe	Discontinue PEGASYS permanently	Obtain immediate psychiatric consultation	Psychiatric therapy necessary		

All patients should be treated pre-emptively with anti-depressants and monitored as above with dose reductions and further intervention as outlined in the table above.

4.3.6 *Other*

For any other grade >3 toxicities, dose adjust PEG-IFN as per guidelines for cytopenias with ANC $\geq$0.5 <0.75 or Plts $\geq$25 <50.

4.4 *Donor lymphocyte infusions*

4.4.1 *Donors*

Donors are assessed and selected as per Institutional guidelines.

Suitable stem cell donors are restricted to the original sibling or volunteer unrelated donor. HLA mismatched donors are not excluded.

4.4.2 *Donor lymphocyte infusions*

DLI may be undertaken in patients without significant GVHD (grade II-IV GVHD or progressive chronic GVHD) after day +35 if indicated. Patients requiring immune suppression greater than 0.5mg/kg of prednisone or equivalent will NOT progress to DLI.

Donor lymphocytes will be collected by steady state leucopheresis from the donor and stored in aliquots (x4). These will be administered without GVHD prophylaxis in a dose escalating regimen at 4 week intervals, depending on re-assessment of chimerism status, GVHD, and / or disease response at 4 weeks after each DLI. Patients may only progress to the next dose level in the absence of significant PRIOR OR CURRENT GVHD (grade II-IV).

Initially $3x10^6$ CD3+ cells/kg recipient body weight will be infused, progressing sequentially to$1x10^7$ CD3+ cells/kg, then $3x10^7$ CD3+ cells/kg, then $1x10^8$ CD3+ cells/kg recipient body weight

4.5 *Supportive care guidelines*

All supportive care is as per Institutional guidelines. A summary is detailed below.

4.5.1 *Definitions of count recovery after FLAG*

Definitions for resolution of cytopenias post FLAG are:

- First day of neutrophil count post nadir of >$1.0x10^9$/L on 3 consecutive days
- First day of platelet count post nadir of >$20x10^9$/L (unsupported) on 5 consecutive days

4.5.2 *Bone marrow assessments or CT restaging for lymphoma*

- Cohort A and B: Cohort A - Pre chemotherapy Cohort B – pre Peg-IFN
- Cohort A: following FLAG/chemotherapy, pre peg-IFN
- Cohort A and B: After D+35 of peg-IFN if DLI is indicated
- Cohort A and B: D+90, D+180, D+360, D+720
- When required to distinguish cause of cytopenias (i.e. disease vs. peg-IFN).

4.5.3 *Graft versus host disease prophylaxis*

No GVHD prophylaxis is to be administered prior to study entry.

4.5.4 *Graft versus host disease treatment*

GVHD will be assessed and graded according to the Seattle criteria (see Section 7). Whenever possible, histological biopsies will be undertaken to confirm diagnosis of GVHD. Patients with moderate to severe (grade II-IV) acute GVHD will be treated with methylprednisolone (or equivalent) at 1-2 mg/kg/day for 2 weeks, with steroid tapering occurring over the subsequent 2-4 months, as per Institutional guidelines. Patients not responding after two weeks or progressing will be treated with CsA or tacrolimus plus prednisolone 1-2 mg/kg/day, as per Institutional guidelines. Second line therapy for refractory GVHD is left to the discretion of the Investigator.

4.5.5 *Blood product support*

All patients will receive irradiated blood products which have undergone pre-storage leucodepletion.

Only *Cytomegalovirus* (CMV) seronegative recipients with CMV negative donors will receive CMV seronegative blood products. All other patients will receive (filtered) CMV irrelevant blood products.

Packed red cells will be given to maintain haemoglobin concentrations above 80-90g/L, and platelet transfusions to maintain platelet counts above 20 $x10^9$/L.

Management of ABO and Rh mismatch between donor and recipient is as per Institutional guidelines.

4.5.6 *Infection prophylaxis*

All patients receive antifungal prophylaxis with Fluconazole 200mg daily from study entry.

Cotrimoxazole is administered after stable engraftment for *Pneumocystis carinii* prophylaxis. Patients allergic to sulfur agents will receive monthly nebulized pentamadine (300mg) for *P. carinii* prophylaxis.

For CMV prophylaxis, all seropositive patients (or seronegative recipients of a seropositive graft) receive valaciclovir prophylaxis 500mg BD PO. CMV surveillance is performed weekly with quantitative Polymerase Chain Reaction (qPCR), with pre-emptive therapy with ganciclovir to be initiated in the event of 2 consecutive positive qPCR assays.

Fluconazole, cotrimoxazole and valaciclovir administration is continued until 1 month after cessation of all immunosuppressive therapy.

4.5.7 *IVIG*

Intravenous immunoglobulin (0.4mg/kg/dose monthly) will be administered to patients with recurrent infections, as per Unit policy.

4.5.8 *G-CSF*

Patients will receive routine growth factor (G-CSF 5mcg/kg) from day +6 following FLAG chemotherapy until ANC recovery. G-CSF is permitted to treat chronic neutropenia in the setting marrow infiltration with non-myeloid malignancies OR peg-IFN-dependent marrow suppression.

5. PHARMACEUTICAL INFORMATION

5.1 *Investigational agent*

Pegasys® is an interferon-2alpha protein produced by recombinant DNA technology within E coli and conjugated to a polyethylene glycol chain (PEG) molecule of 40 kilodaltons. The molecule belongs to the type I interferon class of cytokines and exerts effects by specific binding to its cognate receptor which is widely expressed. Receptor activation results in intracellular signaling and the rapid transcription of genes that control cell proliferation and immune modulation.

Peg-IFN (either Pegasys® or Pegatron®) is currently approved for the treatment of hepatitis B and C alone or in combination with ribavirin. It has been administered to over 5,000 patients in clinical trials. Pegasys® is the formulation used in this study due to its availability without Ribavirin (which is an antiviral not relevant to the anti-leukemia effect of peg-IFN being exploited in this study) and its dose flexibility.

5.2 *Administration*

Pegasys® is available in a 135mcg or 180mcg prefilled syringe and doses of 45mcg, 90mcg, 135mcg or 180mcg can be injected subcutaneously once weekly.

5.3 *Toxicities*

Adverse events attributable to peg-IFN from studies treating hepatitis B/C included:

Common toxicities (incidence ≥1:100)

a. Flu-like symptoms (up to 50%)

Flu-like side effects are a common and predictable side effect. Symptoms include dizziness (89%), fatigue ((50%), headache (44%), myalgia (38%), arthralgia (32%),

fevers (30%), rigors (24%), lack of strength/generalized weakness (22%), injection site reactions (redness, pain, swelling -16%), decrease appetite (8%) and back pain (9%).

Treatment with paracetamol may help reduce symptoms but serious infection must always be considered and excluded when medically indicated. All patients in this study receive routine screening for opportunistic infection as part of their routine pre-HPCT work-up, and during the transplant itself, prophylaxis with norfloxacin 400mg BD PO during the initial cytopenic period, as well as aciclovir / valaciclovir for CMV prophylaxis (also active in prophylaxis against Herpes zoster), fluconazole for fungal prophylaxis and cotrimoxazole for *P. carinii* prophylaxis.

b. Psychiatric disorders (up to 35%)

Serious psychiatric disorders are common and monitoring requires special vigilance. These include Insomnia (35%), depression (26%), irritability (27%) and anxiety (10%). Given that all patients with relapsed leukaemia will be at increased risk of depression it is recommended that anti-depressants are administered routinely to patients on trial.

There are special recommendations for dose reduction and stopping based on psychiatric side effects (see section 4.2).

c. Dermatological

Alopecia (20%), pruritis (16%), skin rashes (14%), and dry skin (11%) have been described during Pegasys® administration.

d. Gastrointestinal

Nausea (32%), diarrhoea (19%), anorexia (16%), vomiting (12%), abdominal pain (9%) and indigestion (9%) have been described at greater frequency during Pegasys® administration than controls.

e. Respiratory

Cough (14%), dyspnea (14%) and pharygitis (9%) have been reported.

f. Haematological

Bone marrow suppression manifesting as anaemia (Hb < 100g/L) was reported in 14% of patients, neutropenia (ANC < 0.5 x 10^9/L) in 4%, thrombocytopenia (<50 x 10^9/L) in 5%, severe lymphopenia (< 0.5 x 10^9/L) in 5% of patients.

Recommendations for Pegasys® dose reductions and stopping based on marrow suppression unrelated to marrow infiltration are outlined in section 4.2.

g. Autoimmune conditions

Since peg-IFN is an immune modulator, exacerbation of autoimmunity is possible and abnormalities in thyroid function (4%) may occur in particular. Other autoimmune disorders described include myositis, hepatitis, psoriasis, rheumatoid arthritis, nephritis and SLE.

Thyroid function should be monitored every 3 months and treated appropriately. If treatment is ineffective, consideration should be made to reducing and/or stopping peg-IFN.

h. Elevated triglycerides

Elevation in random triglycerides to >4.5 mmol/l occurred in 20% and >11.3 mmol/l occurred in 2% of patients with hepatitis C treated with Pegasys®. Lipids will be monitored 3 monthly.

i. Immunogenicity.

Development of anti-peg-IFN antibodies occurred in 9% of patients being treated for hepatitis C and 29% of patients treated for hepatitis B although the significance of this remains unknown.

2. *Uncommon toxicities (incidence <1:100)*

a. Hypersensitivity reactions (including anaphylaxis)

Clinically significant hypersensitivity reactions and / or anaphylaxis have occurred but are uncommon (incidence < 0.1%)

b. Opthalmologic reactions

Retinopathy including retinal haemorrhages, cotton wool spots, optic neuropathy and retinal artery or vein obstruction may occur which has been associated with loss of vision. All patients with pre-existing retinal disorders (e.g. diabetic or hypertension retinopathy) should receive 3 monthly eye reviews. Peg-IFN should be stopped immediately in the setting of decreased or loss of vision.

c. Liver function abnormalities

Increases in ALT have been described in patients with hepatitis B that are thought to represent immune-mediated pathology against the virus. In the context of this trial increases in LFT should be assumed to reflect GVHD and dose reduction and stopping rules in these circumstances are outlined in section 4.2.

d. Respiratory abnormalities

Dyspnea, pulmonary infiltrates, pneumonia, bronchiolitis obliterans, pneumonitis, pulmonary hypertension and sarcoidosis may be induced of aggrevated by IFN. IFN should be stopped in patients developing pulmonary infiltrates or respiratory function impairment until the cause can be evaluated.

e. Cardiovascular abnormalities

Hypertension, SVT, arrhythmias, chest pain and myocardial infarction have been observed in patients receiving peg-IFN and caution should be used in treating patients with pre-existing heart disease.

f. Cerebrovascular abnormalities

Ischemic and haemorrhagic cerebrovascular events have been observed in patients receiving peg-IFN however a cause and effect relationship has been difficult to establish.

g. Colitis

Ulcerative and haemorrhagic/ischemic colitis, sometimes fatal has been observed within 3 months of starting therapy with peg-IFN. Abdominal pain, bloody diarrhoea and fever are typical manifestations. Peg should be stopped immediately in this

setting and patients evaluated for GVHD (e.g. with colonoscopy). Colitis related to peg-IFN usually resolves within 1-3 weeks of ceasing therapy.

h. Pancreatitis

Pancreatitis, sometimes fatal has occurred in patients receiving peg-IFN and ribavirin. Peg-IFN should be stopped immediately if symptoms or signs suggest pancreatitis.

6. STUDY CALENDAR

Evaluations to be conducted include:

6.1 *Screening evaluations*

6.1.1 *Routine evaluations* (as per Institutional guidelines)

- Medical assessment: including relevant medical history, physical examination, ECOG, vital signs, weight.
- Full re-staging of underlying disease
- Other blood investigations: FBE; E/LFT, coagulation profile; thyroid function, lipids, pregnancy testing (females only)

6.1.2 *Study-specific evaluations / procedures*

- Review of study inclusion / exclusion criteria
- Written informed consent

6.2 *During / after study*

For study purposes, patients will be medically assessed weekly from the commencement of Peg-IFN until day 180 and monthly from day 180 to 1 year then finally at 2 years. Medical assessment will consist of physical examination, vital signs, weight, and toxicity assessment, including GVHD assessment and assessment of adverse events. This information must be clearly documented in the patient's medical record. All medical assessments prior to day 180 must be performed at RBWH.

Full blood examination (FBE) and electrolyte and liver function testing (ELFT) will be performed at each medical review. Fasting lipid profile including serum cholesterol and triglyceride levels and thyroid function will be performed D90 , D180, D280, D360, CMV monitoring (qPCR) and other routine post-transplant assessments will be performed as per Institutional guidelines (see Supportive Care Guidelines).

GVHD will be assessed and graded according to the Seattle criteria (see Section 7). Whenever possible, histological biopsies will be undertaken to confirm diagnosis of GVHD.

Study-specific evaluations required include:

1. Reassessment of remission status of underlying disease by BMAT/ or CT restaging for lymphoma :
   - Cohort A and B – cohort A - Pre chemotherapy. cohort B - pre Peg-IFN
   - Cohort A - following FLAG/ chemotherapy, pre peg-IFN
   - Cohort A and B - before DLI if indicated
   - Cohort A and B - at 3, 6, 12 and 24 months

Study-specific assays to be undertaken at QIMR:

ONLY IN PATIENTS NEGATIVE FOR HepBsAg, Hep C Ab, HIV Ab.

All patients are routinely screened and informed of Hep B and Hep C results regardless of study repeat Hepatitis B and C serology and HIV status if not performed within 6 months of screening. For the purpose of this study, we are avoiding research staff handling potentially viraemic samples. In this case patients will be eligible for treatment on the study, but immunological endpoints will be not be measured at QIMR. Peg-Interferon is a standard treatment for Hep B and Hep C.

- Measurement of plasma and cell derived cytokine levels at days:

  Cohort A – D1 (Pre-Peg-IFN), D35, D60, D90, D120, D150, D180, D210, D240, D270, D360 and D720.

  Cohort B – D1 (Pre-Peg-IFN), D35, D60, D90, D120, D150, D180, D210, D240, D270, D360 and D720.

  These assays require 30 mls of blood collected into heparin tubes.

- Numbers and activation status of effector and regulatory T cell subsets will be determined by flow cytometry at the same time points. This requires an additional 30 mls of blood collected into heparin tubes at these time points.

6.2.1 *Study Schema – Cohort A (Haematological Relapse)*

	Screening	**Pre peg-IFN**	**D1** – D100 (± 3 day)**	**D100 – D180 (± 3 days)**	**6 - 12months (± 14 days)**	**24 months (± 30days)**
Medical Assessment* ##	X		X (weekly)	X (weekly)	X (monthly)	X
ECOG*	X		X (weekly)	X (weekly)	X (monthly)	X
Vital signs*	X		X (weekly)	X (weekly)	X (monthly)	X
Weight*	X		X (weekly)	X (weekly)	X (monthly)	X
	X	X	X	X	X	X
Disease restaging	(pre chemotherapy)	following chemotherapy -pre Peg-IFN	D35% (+/- 14 days) D90(+/-14 days)	D180(+/-14 days)	D360	D720
Written Informed Consent	X					

Review Inclusion/ Exclusion Criteria	X					
Adverse Events[$]			X	X	X[$]	
Thyroid function / fasting lipids	X		X D90	X D180	X D270 & D360	
GVHD scoring	X		X (weekly)	X (weekly)	X (monthly)	X
Serum cytokine, T cell subsets [$$]		X D1	X D35, D60, D90	X, D120,D150, D180	X D210, D240, D270, D360	X D720

6.2.2 *Study Schema – Cohort B (Non- Haematological Relapse)*

	Screening	**D1** ** **– D100** **(± 3 day)**	**D100 – D180** **(± 3 days)**	**6 - 12months** **(± 14 days)**	**24 months** **(± 30days)**
Medical Assessment* ##	X	X (weekly)	X (weekly)	X (monthly)	X
ECOG*	X	X (weekly)	X (weekly)	X (monthly)	X
Vital signs*	X	X (weekly)	X (weekly)	X (monthly)	X
Weight*	X	X (weekly)	X (weekly)	X (monthly)	X
Disease restaging	X Pre Peg-IFN	X • D35[%] (+/-14 days) • D90 (+/-14 days) •	X • D180 (+/-14 days)	X D360	X D720
Written Informed Consent	X				
Review Inclusion/ Exclusion Criteria	X				
Adverse Events[$]		X	X	X[$]	

Thyroid function / fasting lipids	X	X D90	X D180	X D270 & D360	
GVHD scoring	X	X (weekly)	X (weekly)	X (monthly)	X
Serum cytokine, T cell subsets [$$]		X D1(prePeg-IFN) D35, D60, D90	X, D120,D150, D180	X D210, D240, D270, D360	X D720

*Patients will be medically assessed prior to entering study, then weekly from Day 1 until day 180, and monthly from day 180 to 1 year and at 2 years post study entry. Medical assessment will include physical examination, ECOG, vital signs, weight, and toxicity assessment including GVHD assessment and assessment of adverse advents (from day 1 to day 180).

[##]FBE and ELFT will be performed at each medical review. Thyroid function and fasting lipid profile including serum cholesterol and triglyceride levels will be performed at study entry and then D90, D180, D270, D360. CMV monitoring (qPCR) and other routine assessments will be performed as per Institutional guidelines.

[$$]Measurement of plasma and cell derived cytokine levels at days:

- Cohort A – D1 (pre Peg-IFN), D35, D60, D90, D120, D120, D150, D180,D210, D240, D270, D360 and D720.
- Cohort B – D1 (pre Peg-IFN), D35, D60, D90, D120, D120, D150, D180,D210, D240, D270, D360 and D720.

These assays require 30 mls of blood collected into heparin tubes. Numbers and activation status of effector and regulatory T cell subsets this requires an addition 30 mls of blood collected into heparin tubes and will be determined by flow cytometry at the same time points.

[**]Day1 = commencement of peg-IFN – without significant acute GVHD (Grade II-IV) or progressive chronic GVHD, no immune suppression except Prednisone<0.5mg/kg

[%]If DLI indicated

[^^] Definitions for resolution of cytopenias post FLAG are:

- First day of neutrophil count post nadir of >1.0x10^9/L on 3 consecutive days
- First day of platelet count post nadir of >20x10^9/L (unsupported) on 5 consecutive days

[$]AE & SAE commence D1 and finish ideally within 28 days after peg-IFN completed/stopped

7. MEASUREMENT OF EFFECT

7.1 *Endpoints*

7.1.1 *Primary endpoint*

The primary endpoint is overall survival at 2 years.

7.1.2 *Secondary endpoints*

7.1.2.1 Disease Response

7.1.2.2 Incidence of GVHD.

7.1.2.3 Treatment related mortality.

7.1.2.4 Incidence and severity of peg-IFN toxicity

7.2 Definition of endpoints

7.2.1 *Overall Survival*

OS is measured from study entry until death from any cause.

7.2.2 *Disease Response*

Maximal response as determined by bone marrow examination. Complete Remission (CR), Partial Remission (blasts 5-15%, >75% reduction in disease if lymphoma/myeloma), Stable Disease (SD), Progressive Disease (PD).

7.2.3 *Graft versus host disease (GVHD)*

Acute GVHD will be assessed and graded according to the Seattle criteria (see Tables below).[56]

STAGE	**SKIN**	**LIVER**	**GUT**
0	No rash	Bilirubin < 34 umol/l	diarrhoea <500ml / day
1	Rash <25% of body surface	34-51 umol/l	diarrhoea 500-1000 ml/day
2	Rash 25-50% of body surface	51-102 umol/l	diarrhoea 1000-1500ml / day
3	Generalised erythroderma	102-255 umol/l	diarrhoea >1500ml / day
4	Bullae and desquamation	>255 umol/l	Pain or ileus

GRADE	**SKIN**	**LIVER**	**GUT**	**CLINICAL PERFORMANCE**
I (mild)	1 to 2	0	0	Normal
II (moderate)	1 to 3	1	1	Mild decrease
III (severe)	2 to 3	2 to 3	2 to 3	Marked decrease
IV (life threatening)	2 to 4	2 to 4	2 to 4	Incapacitated

Chronic GVHD is defined as GVHD occurring beyond day +100 post Haematopoetic Progenitor Cell Transplant (HPCT)

Chronic GVHD will be assessed and graded according to the Seattle criteria (see Table below).[57] Whenever possible, histological biopsies will be taken to confirm diagnosis of GVHD.

LIMITED CHRONIC GVHD	**EXTENSIVE CHRONIC GVHD**
Either or both:	Either:
1 Localised skin involvement	1 Generalised skin involvement; or
2 Hepatic dysfunction due to chronic GVHD	2 Localised skin involvement and/or hepatic dysfunction due to chronic GVHD, plus:
	A Liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis; or
	B Involvement of eye: Schirmer's test with less than 5 mm wetting; or
	C Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy; or
	D Involvement of any other target organ

7.2.2 *Treatment related mortality (TRM)*

Any deaths not directly attributable to underlying disease will be included as TRM. This includes all GVHD and infection related deaths.

7.3 Adverse events (AEs)

7.3.1 *Defintion*

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product which does not necessarily have to have a causal relationship with this treatment.

7.3.2 *Serious adverse events (SAE)*

An AE that matches the criteria defined below will be recorded as an SAE:

- Fatal
- Life threatening
- Results in a persistent or significant disability or incapacity
- Requires hospitalization or prolongation of existing hospitalization
- Results in a congenital malformation
- Medically significant event requiring medical or surgical intervention to prevent the above outcomes listed

All SAEs will be reported to the Principal Investigators and the RBWH Human Research Ethics Committee within 24hrs of an Investigator becoming aware of the event.

7.3.3 *Grading of AEs*

All AEs will be graded as per Common Terminology Criteria for Adverse Events (CTCAE), version 4:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

7.3.4 *Reporting of AE and SAEs*

AEs and SAEs will be reported from the first day of the participant receiving study drug (peg-IFN) ideally until 28 days after study drug completed.

Due to the expected toxicity of treatment in this very high risk population, only > grade 3 toxicities will be recorded as AEs.

Since all patients are expected to develop grade 4 haematological toxicity (neutropenia, thrombocytopenia) due to the FLAG chemotherapy and/or marrow involvement by haematological malignancies, these will not be reported as AEs / SAEs.

8. STATISTICAL CONSIDERATIONS

8.1 *Study design / primary endpoint*

The trial uses a single arm phase I / II design with the primary endpoint incidence of overall survival at 2 years after study entry.

Analysis of the historical allogeneic HPCT cohort receiving FLAG chemotherapy at RBWH demonstrates that overall survival in this cohort is 7% at two years. To detect an increase to 40% with 80% power and a two-sided significance level of 5%, a sample size of 29 patients is required.

8.2 *Accrual rate*

Based on the number of allogeneic HPCT performed at RBWH (approximately 80 per year) with 15-20 relapses per annum, it is expected to recruit 10 patients per year, with enrolment completed within 36 months. Patients who withdraw or are withdrawn from the trial prior to commencement of Peg-Interferon will be replaced by new eligible patients

8.3 Secondary *endpoints*

All secondary endpoints will be examined and reported in a descriptive manner.

Survival curves (PFS; OS) will be produced using the Kaplan-Meier method. PFS and OS analysis will be performed at 24 months post enrolment onto the study.

8.4 *Stopping rules*

Interim analyses will be performed after each cohort of 10 patients has passed the 6 month follow-up mark.

1. Unacceptable mortality or absence of efficacy. The primary endpoint is survival at two years. The study will be closed at the interim time-point if the lower confidence interval for survival is below 10%.
2. Unacceptable Peg-IFN toxicity (> grade 3 toxicity as per CTCAE v. 4 in ≥ 50% of recipients unrelated to GVHD).
3. Unacceptable GVHD (severe steroid refractory acute GVHD (grade III-IV) in ≥ 50% of recipients).

8.5 *Independent External Review*

Prof Liz Powell (Liver Unit, PA Hospital, Brisbane) and Dr Paul Kubler (Dept of Pharmacy, RBWH, Brisbane) will act as independent external reviewers during the course of the study. They will review the accruing data, including adverse events, after each cohort of 10 patients after each cohort of 10 patients have completed 6 months of study.

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APPENDIX A

Eastern Cooperative Oncology Group (ECOG) performance status scale

ECOG Scale	Performance Status
0	Fully active, able to carry out all pre-disease performance without restriction.
1	Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work.
2	Ambulatory and capable of all selfcare, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completed disabled. Cannot carry out any selfcare. Totally confined to bed or chair.
5	Dead

NOTE:

ECOG 0 corresponds to Karnofsky performance status of 100 - 90

ECOG 1 corresponds to Karnofsky performance status of 80 - 70

ECOG 2 corresponds to Karnofsky performance status of 60 - 50

ECOG 3 corresponds to Karnofsky performance status of 40 - 30

ECOG 4 corresponds to Karnofsky performance status of	20 - 10
ECOG 5 corresponds to Karnofsky performance status of	0