

Royal Brisbane and Women's Hospital/Queensland Institute of Medical Research

A phase I / II study of humanized anti-IL-6 receptor antibody Tocilizumab (TCZ) to prevent development of acute graft versus host disease (GVHD) post HLA-matched 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 and non-malignant conditions. The main clinical limitation of HPCT is development of graft versus host disease (GVHD), a complex immune reaction involving activation and proliferation of donor-derived T cells which induce recipient organ / tissue damage via a range of immune effector mechanisms. It has been recognized for several years that pro-inflammatory cytokines, including TNF α and interleukin-1 (IL-1) are important mediators of GVHD. Recent preclinical models of HPCT have also confirmed the central role of IL-6 in GVHD pathogenesis; use of neutralizing anti-IL-6 receptor monoclonal antibody (anti-IL-6R mAb) significantly reduces GVHD severity and improves survival in preclinical models but importantly, also maintains graft versus malignancy (GVM) responses.¹ A humanized anti-IL-6R mAb Tocilizumab (TCZ) has been extensively studied in humans and is now approved for clinical use in rheumatoid arthritis.

Aims: To determine the safety and efficacy of TCZ in preventing development of acute GVHD in HLA-matched allogeneic HPCT after myeloablative or reduced intensity conditioning (RIC).

Study Design: Phase I / II study design.

Population: Eligible patients are those undertaking T cell replete HLA-matched sibling or volunteer unrelated allogeneic HPCT. Exclusion criteria include elevated liver enzymes (>3.0 x ULN).

Treatment: HPCT conditioning regimens will include both myeloablative (e.g. cyclophosphamide / Total Body Irradiation) and reduced intensity (e.g. fludarabine / melphalan and fludarabine / busulphan) protocols. TCZ will be administered at a standard dose of 8mg/kg at day -1 of transplantation. All patients will continue to receive standard GVHD prophylaxis with cyclosporine and methotrexate, as per Institutional guidelines. Supportive care, including antibiotic prophylaxis and nutritional support will also be administered as per Institutional guidelines.

Outcomes: The primary endpoint is incidence of grade II-IV acute GVHD at day +100 post HPCT. Secondary endpoints include serum IL-6 levels post-HPCT, engraftment rate and time to engraftment, rate of infection including *Cytomegalovirus* (CMV) reactivation, incidence of liver toxicity including veno-occlusive disease (VOD), incidence of chronic GVHD, transplant related mortality (TRM), progression-free (PFS) and overall survival (OS).

Assessment: GVHD will be staged and graded according to standard Seattle criteria. GVHD assessment, assessment of infective complications, liver toxicity, PFS and OS will occur at 1, 2, 3, 6, 9, 12 and 24 months post transplantation. Cytokine and cellular responses will be measured regularly up until one year post transplant. TCZ levels may be measured regularly up until one month post transplant.

Statistics: Analysis of a historical HPCT patient cohort at RBWH demonstrates that incidence of grade II-IV acute GVHD is approximately 60%. To detect a 33% reduction in incidence of grade II-IV GVHD, with 80% power and a two-sided significance level of 5%, a sample size of 48 patients is required.

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Feasibility: The trial will be conducted locally with an estimated 32 patients recruited per year. Enrolment is expected to be completed by 18 months.

OBJECTIVES

This study aims to assess the safety and efficacy of the humanized anti-IL-6 receptor monoclonal antibody (anti-IL-6R mAb), Tocilizumab (TCZ), in preventing development of acute graft versus host disease (GVHD) after HLA-matched allogeneic haematopoietic progenitor cell transplantation (HPCT).

1. BACKGROUND

1.1 GVHD and 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 malignancy" (GVM) 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 GVM immune responses enables the potential to limit post-transplant morbidity / mortality whilst maintaining the therapeutic potential of HPCT.

The overall incidence of clinically significant (grade II-IV) acute GVHD following T cell replete HPCT (including at the RBWH Bone Marrow Transplant Unit) is 60%.^{2,3} Once established, complete responses occur in less than 40% of patients with acute GVHD and over half of patients refractory to steroids will die within 6 months.^{4,5} These poor responses to therapy and high mortality rates highlight the need to develop more effective therapies for preventing acute GVHD.

The standard approach to prevent development of GVHD is administration of daily cyclosporine (CsA) and methotrexate (MTX) on days +1 (15mg/m²), +3, +6 and +11 (10mg/m²). Attempts to improve on standard "CsA + MTX" prophylaxis to reduce GVHD incidence have included use of CsA substitutes (such as tacrolimus), use of MTX substitutes (such as mycophenolate mofetil [MMF]), addition of a 3rd immunosuppressive agent (such as corticosteroids or sirolimus) and removing T cells from the graft ("T cell depletion"). In general, none of these alternative approaches to GVHD prophylaxis have improved upon standard CsA + MTX, with any reduction in GVHD incidence offset by either increased infectious or other treatment-related complications, or increased relapse rates of the underlying (malignant) disease.⁶⁻¹⁵

1.2 GVHD Biology

Acute GVHD is generally defined as occurring within three broad stages:¹⁶

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.¹⁷⁻¹⁹ 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.

IL-6 has recently been recognized to play an important role in the pathogenesis of acute GVHD although some controversy exists over the mechanism of action. There have been two major preclinical studies examining this, one from QIMR and University of Michigan, USA, the other from Milwaukee, USA. Both demonstrate similar (high) levels of protection from GVHD following IL-6 inhibition early after HPCT.^{1,20} However, while one study suggested the effects of IL-6 inhibition were the result of alterations in IL-17 and regulatory T cell development,²⁰ we instead demonstrated that IL-6 was directly cytopathic to recipient tissue (like TNF). The fact that GVM effects are unaffected by IL-6 inhibition (in contrast to TNF inhibition²¹) suggests that direct cytopathic effects on target tissue rather than indirect effects on adaptive immunity are the likely dominant pathogenic effect of the cytokine.

1.3 IL-6

IL-6 is a member of the gp130 cytokine family that includes IL-11, IL-27, IL-31, leukaemia inhibitory factor, oncostatin M and cardiotrophin-1. These cytokines all require the gp130 cytokine receptor subunit to signal. However only IL-6 and IL-11 signal via the induction of gp130 homodimers after interaction with the relevant cytokine specific subunit (the IL-6R α subunit in the case of IL-6). The distribution of the IL-6R α receptor subunit is relatively limited in distribution and includes monocytes, macrophages, neutrophils, T cells and hepatocytes. In contrast, the gp130 subunit is ubiquitously expressed.^{22,23} The IL-6R α can be cleaved to a soluble form by proteolytic cleavage mediated by metalloproteases. In addition, alternatively spliced IL-6R α proteins can be generated that lack membrane anchoring domains and exist in soluble form only. Importantly the soluble IL-6R α acts as a cytokine agonist and IL-6 can exert effects via classical or *trans*-signalling.

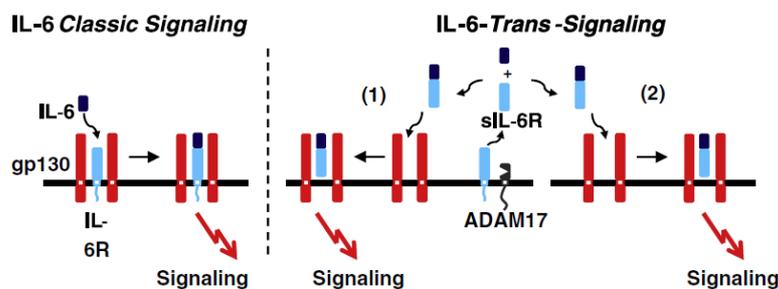


Fig. 1. Classic- and *trans*-signaling of IL-6. Cells that express both gp130 and the IL-6R are responsive to IL-6 (*classic signaling*). However, cells that express only gp130 can be activated by the IL-6/sIL-6R complex. Activation of cells by the IL-6/sIL-6R complex is termed *trans*-signaling. The sIL-6R is generated by proteolytic cleavage of the membrane bound precursor. The sIL-6R binds IL-6 with comparable affinity as the membrane bound form and mediates gp130 activation in an (1) autocrine or (2) paracrine manner.

Classical signalling involves IL-6 signalling via membrane receptors and can exert anti-inflammatory properties via stat3 activation. In contrast the soluble IL-6R α -IL-6 complex can bind to gp130 homodimers and signal in *trans* within cells that do not normally express the IL-6R α . This effect is inflammatory and in particular is involved in immune cell activation. This concept of signalling is shown in Figure 1 (taken from²²). In most inflammatory settings the *trans* signalling dominates and inhibition of the IL-6R α with mAb inhibits both signalling pathways.

1.4 Role of IL-6 in GVHD

1.4.1 Clinical data

An increase in systemic IL-6 levels early after HPCT was first described in the early 1990's.^{24,25} This increase in IL-6 was demonstrated using older bioassays where specificity and sensitivity are inferior to current flow cytometry based assays. Interestingly, these studies demonstrated elevations in IL-6 that correlated with infectious or toxic complications (pneumonitis, veno occlusive disease) after HPCT but were conflicting in regards to any association with GVHD.^{24,25} This likely reflects the small study size and their heterogeneity with regard to both conditioning (cyclophosphamide / Total Body Irradiation [TBI], busulphan / cyclophosphamide and cyclophosphamide / Anti-Thymocyte globulin) and disease states (i.e. chronic *versus* acute leukaemia, lymphoma and aplastic anaemia).

The RBWH and QIMR have now significantly extended these findings using highly sensitive cytokine bead-based assays. Plasma IL-6 levels were measured in a consecutive cohort of 17 patients undertaking HLA-matched sibling HPCT. Patients were sampled at baseline and repeatedly for a period of up to one year. IL-6 levels were significantly and markedly elevated early after HPCT. This increase was detectable at day 0, after conditioning and before transplantation, clearly demonstrating that conditioning itself invokes IL-6 secretion. However, thereafter, IL-6 levels rose markedly relative to baseline (day -7; **Figure 2**), peaking 7 days after HPCT. Importantly, IL-6 levels remained elevated at day +14 but returned to baseline by day +30. No other consistent rises in other cytokines were seen, including IL-2, interferon-gamma or TNF (data not shown). Patients who received myeloablative conditioning (cyclophosphamide + TBI) showed a trend to higher IL-6 levels than those receiving reduced intensity conditioning (fludarabine + melphalan; **Figure 3A**). Strikingly, IL-6 levels at day +7 were highest in patients who subsequently developed acute GVHD suggesting that this cytokine both precedes and predicts for the subsequent development of acute GVHD (**Figure 3B**). A relationship between GVHD and elevations in other inflammatory cytokines was not seen.

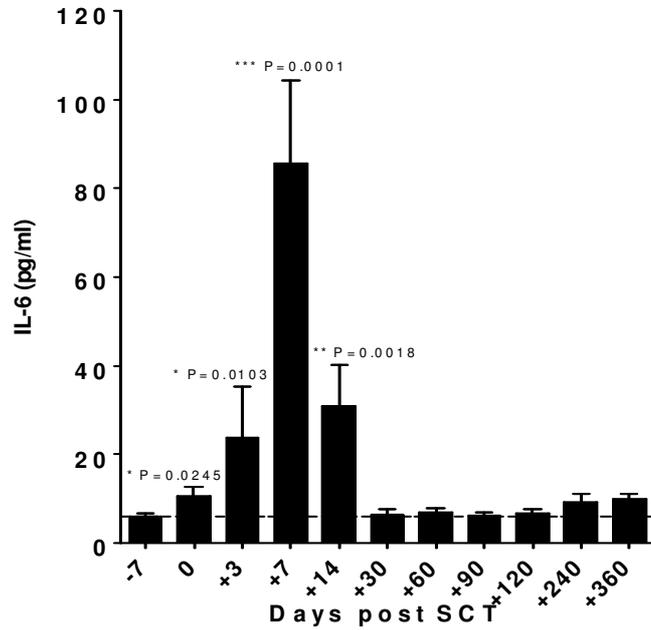


Figure 2: Plasma IL-6 levels in cohort of 17 patients undertaking HLA-matched sibling HPCT at RBWH

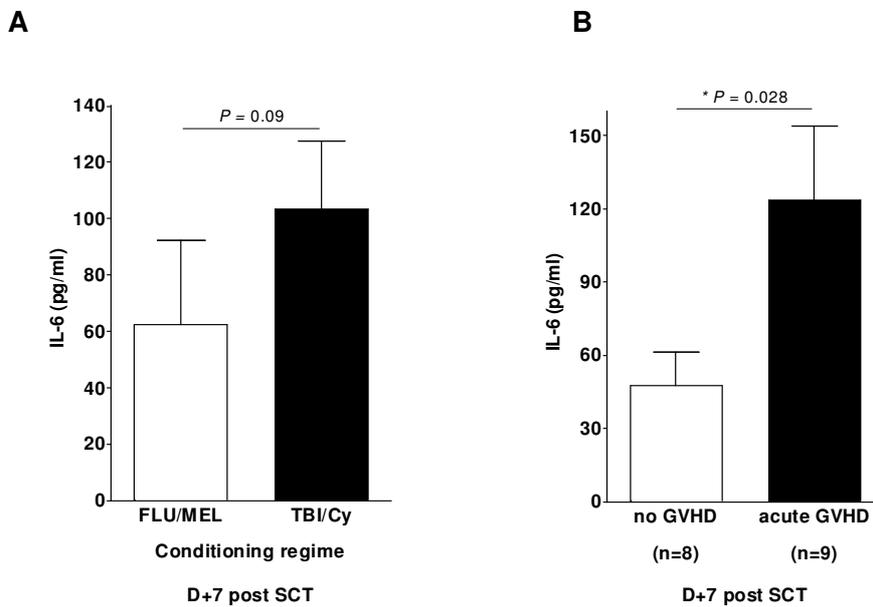


Figure 3: D+7 plasma IL-6 levels in cohort of 17 patients undertaking HLA-matched sibling HPCT at RBWH; **A:** stratified according to conditioning regimen (fludarabine / melphalan vs cyclophosphamide / TBI); **B:** stratified according to development of GVHD

Two publications to date have reported IL-6 inhibition with the monoclonal anti-IL-6R antibody TCZ for the treatment of severe, steroid refractory GVHD. A case report of a patient with severe acute GVHD of the gastrointestinal tract (GIT) demonstrated a complete response to TCZ without significant toxicity.²⁶ A second small case series demonstrated responses in 4 of 6 patients (2 complete responses and 2 partial responses) with severe steroid refractory acute GVHD treated with 8mg/kg of TCZ every 3-4 weeks.²⁷ While these studies suggest that TCZ has efficacy in the treatment of established severe GVHD they do not recapitulate the dramatic protection from GVHD seen in preclinical studies when IL-6 receptor (IL-6R) inhibition is utilized early after transplantation as prophylaxis rather than treatment.

1.5 Tocilizumab

TCZ is a humanized anti-IL-6R mAb which binds to both soluble and transmembrane IL-6R, inhibiting IL-6 binding and thus blocking IL-6 signalling through both receptors. TCZ is specific for IL-6R and does not block signalling of other IL-6 family cytokines. Single dose pharmacokinetic studies in animals and humans have shown that clearance of TCZ is slow, with a half-life of 6 to >9 days.

TCZ has been extensively studied in rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis, with >3600 patients enrolled into several large randomized phase III studies comparing TCZ as either monotherapy or combination therapy with disease modifying anti-rheumatic drugs (DMARDs; typically methotrexate) to placebo as treatment for refractory arthritis. These trials established the efficacy of TCZ in the therapy of RA refractory to DMARDs and other biological agents, with >50% of patients experiencing improvement in general health status via reduction in clinical symptoms and normalization of inflammatory markers. A summary of these trials can be found in references 28-30.²⁸⁻³⁰ TCZ is now approved by the Therapeutic Goods Administration (TGA) for use in RA in Australia.

The standard dose schedule of TCZ in RA (and other systemic inflammatory disorders) is 8mg/kg (up to a maximum of 800mg) administered as an intravenous (IV) infusion over 60mins, repeated every 4 weeks. Based on the randomized phase III studies in RA and subsequent follow-up open label extension studies, TCZ appears very well tolerated with low rates of medication discontinuation, overall low rates of adverse events (AE) and no new AEs and / or safety concerns with ongoing / repeated exposure and longer-term follow-up. The toxicity profile of TCZ is very manageable, and includes infections (most notably skin and soft tissue), increases in serum cholesterol, transient decreases in neutrophil count and (typically transient) abnormal liver function tests (see Section 5.3).²⁸⁻³⁰

TCZ has also been demonstrated to have significant activity in other systemic inflammatory disorders, including multicentric Castleman's disease and Crohn's disease / inflammatory bowel disease.^{31,32} As described above, TCZ has also been used in HPCT to treat steroid refractory GVHD. In a HPCT setting, TCZ use in the treatment of GVHD appears well tolerated, with significant clinical efficacy and no unexpected toxicities observed (see Section 5.3).^{26,27}

2. STUDY RATIONALE

The standard approach to prevent development of GVHD remains administration of CsA plus MTX. However, this approach still fails to prevent acute GVHD in 50-70% of HPCT recipients.

Thus new strategies to prevent GVHD, ideally that also spare GVM effects, are clearly needed. Our preclinical data demonstrates dramatic protection from GVHD when IL-6 is inhibited in the first two weeks after HPCT. Subsequent clinical data demonstrates that IL-6 is elevated only within the first 30 days after HPCT and IL-6 levels correlate with the development of subsequent acute GVHD. The anti-IL-6R mAb TCZ is now approved for clinical use (in systemic inflammatory arthritis) with a defined and manageable toxicity profile. We therefore plan to use a single dose of TCZ at day -1 to inhibit IL-6 signaling for the first 3-4 weeks after HPCT as an adjunct to standard CsA + MTX as prophylaxis for acute GVHD.

3. PATIENT SELECTION

3.1 Inclusion Criteria

- 3.1.1 Patients undertaking a T cell-replete HLA-matched allogeneic HPCT using either myeloablative or reduced intensity conditioning (see Section 4.1)
- 3.1.2 Age ≥ 18 and < 65 years
- 3.1.3 Life expectancy of greater than 3 months
- 3.1.4 Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 (Karnofsky $\geq 50\%$; see Appendix A)
- 3.1.5 Adequate organ function for allogeneic stem cell transplantation as per Institutional guidelines, defined in Table 1 below:

Table 1

Total bilirubin	$\leq 30 \mu\text{mol/L}$
Serum transaminases (AST / ALT)	$\leq 3.0 \times \text{ULN}$ (i.e. $<$ pre-existing grade 2 toxicity)
Creatinine clearance	$\geq 50 \text{ mL/min/1.73 m}^2$ for patients with creatinine levels above ULN
Left ventricular ejection fraction	$\geq 40\%$
Pulmonary diffusion capacity	$\geq 40\%$ predicted

ULN = upper limit of normal at local institutional laboratory

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3.1.6 HLA-matched sibling donor by typing at HLA-A, B, C and DRB1 loci

3.1.7 HLA- matched volunteer unrelated donor (VUD) by typing at HLA-A, B, C, DRB1 and DQ loci)

3.1.8 Able and willing to provide written informed consent

3.2 Exclusion Criteria

3.2.1 Inadequate organ function for allogeneic stem cell transplantation as per Institutional guidelines, defined in Table 2 below:

Table 2

Total bilirubin	>30µmol/L
Serum transaminases (AST / ALT)	>3.0 x ULN (i.e. >pre-existing grade 1 toxicity)
Creatinine clearance	<50 mL/min/1.73 m ² for patients with creatinine levels above ULN
Left ventricular ejection fraction	<40%
Pulmonary diffusion capacity	<40% predicted

3.2.2 Patients receiving any other investigational agents.

3.2.3 Patients with a past history of solid tumours within prior 2 years (excluding completely excised cutaneous BCC and SCC).

3.2.4 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness / social situations that would limit compliance with study requirements.

3.2.5 Known HIV, HCV and HBV infection.

3.2.6 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.

3.2.7 Patients with a past history of complicated diverticulitis, including fistulae, abscess formation or gastrointestinal (GI) perforation.

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

3.2.9 History of allergic reactions attributed to compounds of similar chemical or biologic composition as TCZ, including known allergies to Chinese hamster ovary cell products or other recombinant human or humanized antibodies.

4. TREATMENT PLAN

4.1 Tocilizumab Administration

TCZ will be administered on an inpatient basis, as a single dose on day -1 of conditioning (day-1). TCZ is administered as an IV infusion over 60mins using a standard infusion set. TCZ is administered at room temperature. Dose is 8mg/kg, up to a maximum dose of 800mg. Due to a lack of physical or biocompatibility studies, TCZ will not be infused with other drugs in the same intravenous line.

Standard pre-medication with paracetamol 1gm orally (PO) and phenergan (promethazine) 12.5mg IV will be administered 15-30mins pre-TCZ infusion to prevent TCZ-related infusion reactions.

4.2 Conditioning protocols

Depending on individual patient diagnosis, age and co-morbidities, patients may undergo HPCT using a variety of different conditioning protocols. Conditioning protocols included in the study consist of both myeloablative and reduced intensity protocols (RIC) as defined below (including TCZ administration at day -1 as part of the study protocol):

4.2.1 Myeloablative conditioning protocols

4.2.1.1 Cyclophosphamide/TBI

Cyclophosphamide	IV 60mg/kg/day	Days -5 to -4
[with Mesna IV 72mg/kg/day; 2gm given 30mins pre-cyclophosphamide, and remainder of dose given as continuous IV infusion over 23 ½ hrs]		
TBI	2Gy BD (8 hours apart)	Days -3, -2 & -1
Tocilizumab	IV 8mg/kg	Day -1 [approximately 12MD, infuse over 60mins; max dose 800mg]

4.2.1.2 Melphalan/TBI

Melphalan	IV 140mg/m ²	Day -4
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TBI	2Gy BD	Days -3, -2 & -1
Tocilizumab	IV 8mg/kg	Day -1 [approximately
	12MD; infuse over 60mins; max dose 800mg]	

4.2.1.3 Busulphan/Cyclophosphamide

Busulphan*	PO 4mg/kg/day in 4 doses	Days -7 to -4
Cyclophosphamide	IV 60mg/kg/day	Days -3 & -2
	[with Mesna IV 72mg/kg/day; 2gm given 30mins pre-cyclophosphamide, and remainder of dose given as continuous IV infusion over 23 ½ hrs]	
Tocilizumab	IV 8mg/kg	Day -1 [approximately
	12MD; infuse over 60mins; max dose 800mg]	

NB: seizure prophylaxis with phenytoin (loading dose of 300mg IV TDS Day -8, then 300mg IV OD Day -7 to Day -3) must also be given with high dose busulphan chemotherapy (alternative anti-seizure prophylaxis is clonazepam 0.5mg BD Day -8 to Day -3)

*If available, IV busulphan (dose equivalent) may be substituted for the oral preparation. Whether oral or IV busulphan is used, area under curve (AUC) monitoring of busulphan levels is recommended as per Institutional guidelines.

4.2.2 Reduced intensity conditioning protocols

4.2.2.1 Fludarabine/Melphalan

Fludarabine	IV 25mg/m ²	Days -7 to -3
Melphalan	IV 120mg/m ²	Day -2
Tocilizumab	IV 8mg/kg	Day -1 [approximately
	12MD; infuse over 60mins; max dose 800mg]	

4.2.2.2 Fludarabine/Busulphan

Fludarabine	IV 40mg/m ² /day	Days -6 to -3
Busulphan*	IV 130mg/m ² /day	Days -6 to -3
Tocilizumab	IV 8mg/kg	Day -1 [approximately
	12MD; infuse over 60mins; max dose 800mg]	

NB: seizure prophylaxis with phenytoin (loading dose of 300mg IV TDS Day -7, then 300mg IV OD Day -6 to Day -2) must also be given with high dose busulphan chemotherapy (alternative anti-seizure prophylaxis is clonazepam 0.5mg BD Day -7 to Day -2)

*Whether oral or IV busulphan is used, AUC monitoring of busulphan levels is recommended as per Institutional guidelines.

4.3 Haematopoietic progenitor cell collection / administration

4.3.1 Donors

Donors are assessed and selected as per Institutional guidelines.

Suitable stem cell donors are either HLA-identical sibling donors, or fully matched volunteer unrelated donors.

HLA matching will be undertaken as per current Institutional and laboratory guidelines as follows:

1. Matched sibling donors will be matched at HLA A, B and C loci using low resolution sequence specific primers (SSP), and at HLA-DRB1 loci using high resolution typing with sequence based typing (SBT) methods (i.e. 8/8 match).
2. Matched unrelated donors will be matched at HLA A, B, C as well as DRB1 and DQ loci using high resolution typing with SBT (i.e. 10/10 match).

Standard HPC mobilization and collection procedure is detailed in Appendix B.

4.3.2 Haematopoietic progenitor cell manipulation

Routine T cell depletion of HPC grafts is not allowed. As per Institutional guidelines, for peripheral blood HPC grafts, no routine red blood cell or plasma depletion is necessary irrespective of ABO matching / mismatching. In the event of bone marrow harvest / collection, red cell and / or plasma depletion of the graft should be undertaken based on ABO matching / mismatching and donor / recipient anti-ABO isoagglutination titres, as per Institutional guidelines.

4.3.3 Haematopoietic progenitor cell reinfusion

As per Institutional guidelines, all patients should receive phenergan (promethazine) 12.5mg IV and paracetamol 1gm PO as pre-medication 30mins prior to HPC reinfusion.

Management of major ABO mismatched HPC is also as per Institutional guidelines, and includes potential use of recipient plasma-exchange, administration of pre-infusion donor group secretor plasma and for bone marrow grafts, red cell depletion.

4.4 Supportive Care Guidelines

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

4.4.1 Graft versus host disease prophylaxis

Standard GVHD prophylaxis is cyclosporine (CsA) and methotrexate (MTX) as per Institutional guidelines (see below).

Cyclosporin A

IV 5mg/kg

Days -1 to +1

Tocilizumab (TCZ)

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	IV 3mg/kg	Day +2 onwards
Methotrexate	IV 15mg/m ²	Day +1
	IV 10mg/m ²	Days +3, +6, +11
Folinic acid	IV 15mg	24hrs post each MTX

CsA is commenced at day -1, as per schedule above. Dosing is then adjusted to maintain trough levels at 140-300 ng/ml, as measured by whole blood high performance liquid chromatography (HPLC). CsA levels will be performed at least twice per week, commencing at day +1. CsA is continued at full dose until day +100. After this time, in the absence of significant acute GVHD, CsA dose is reduced by approximately 10% per 1-2 weeks, with planned cessation of CsA by 6-12 months post-transplantation. Prior to day +100, dose reductions to CsA should only be made if CsA related toxicity is present or levels exceed 300 ng/ml in the absence of toxicity.

Dose adjustments to MTX are also defined as per Institutional guidelines and are indicated in the presence of significant fluid overload and / or organ toxicity (see below). If MTX is unable to be administered (any dose), Mycophenolate mofetil (1gm BD IV or PO) +/- methylprednisolone (1mg/kg/day as per Ruutu protocol¹¹) may be substituted for MTX as GVHD prophylaxis, depending upon relapse risk, GVHD risk and Investigator preference.

i. Fluid overload

In the presence of significant pleural effusions, ascites and / or fluid overload, MTX dose may either be withheld or dose reduced by 50%. If administered in this setting, serum MTX levels should be measured daily from 24 hours post administration, and folinic acid dose increased to 15mg every 6 hours, with folinic acid rescue continued until MTX levels fall below 0.02 mmol/L.

ii. Renal failure

50% dose reduction is indicated with GFR of 10-50ml/min. MTX is withheld if GFR is <10ml/min.

iii. Liver failure

If bilirubin is 50-85 mmol/L, and / or AST > 5x normal, 25% dose reduction is used (i.e. 75% of dose administered). MTX should be withheld if bilirubin is >85 mmol/L.

iv. Significant mucositis

Dose adjustments for mucositis should be discussed with the Investigator. In general, if severe mucositis is present, depending on the severity of mucositis and preference of the Investigator, the day +11 MTX dose may be withheld, administered at full dose, or dose reduced (usually by 50%).

4.4.2 Venocclusive disease (VOD) prophylaxis

All patients will receive standard URSO 500mg BD orally as VOD prophylaxis. Established VOD will be treated with standard dose defibrotide (DFO), as per Institutional guidelines.

4.4.3 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. If feasible, sections of these biopsies may also be assessed for IL-6R expression and / or other cytokine / activation signals by immunohistochemistry (see Section 6). Patients with moderate to severe (grade II-IV) acute GVHD will be treated with methylprednisolone (or equivalent) at 2mg/kg/day for 2 weeks, with steroid tapering occurring over the subsequent 2-4 months, as per Institutional guidelines. Patients with extensive stage chronic GVHD will be treated with CsA plus prednisolone 1mg/kg/day, as per Institutional guidelines. Second line therapy for refractory GVHD is left to the discretion of the Investigator.

4.4.4 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 80-90 g/L, and platelet transfusions to maintain platelet counts above $20 \times 10^9/L$.

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

4.4.5 Infection prophylaxis

All patients receive antifungal prophylaxis with fluconazole 200mg daily with conditioning.

Cotrimoxazole is administered initially during conditioning (until day -1), and recommenced after stable engraftment for *Pneumocystis carinii* prophylaxis. Patients allergic to sulfur agents will receive monthly nebulized pentamidine (300mg) for *P. carinii* prophylaxis.

For CMV prophylaxis, all seropositive patients (or seronegative recipients of a seropositive graft) receive aciclovir 500mg/m² IV TDS during initial cytopenia, then 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.

During the initial cytopenic period, norfloxacin 400mg BD PO is administered as antibacterial prophylaxis (day -8 until development of fever).

4.4.6 Hyperlipidaemia

There is a potential for TCZ to increase serum cholesterol (see Section 5.3). All patients will have fasting lipid profiles performed during pre-HPCT work-up and again at day +100 post HPCT. Persistent rises in serum cholesterol at day +100 will be managed as per Institutional guidelines.

4.4.7 IVIG

Intravenous immunoglobulin will be administered monthly from D-1 for pre-existing hypogammaglobulinaemia as per Institutional guidelines.

4.4.8 G-CSF

Patients will not receive routine post-HPCT growth factors (G-CSF). G-CSF may be administered at the discretion of the Investigator after day +11 MTX administration in the presence of severe mucositis or active infection. G-CSF may also be considered in the setting of persistent neutropenia after day +15 post-HPCT (neutrophil count $< 0.5 \times 10^9/L$).

4.5 Engraftment studies and donor lymphocyte infusions (DLI)

4.5.1 Definitions of engraftment

Peripheral blood engraftment is defined as resolution of cytopenias post HPCT. Definitions for resolution of cytopenias post HPCT are:

- First day of neutrophil count post nadir of $>0.5 \times 10^9/L$ on 3 consecutive days
- First day of platelet count post nadir of $>20 \times 10^9/L$ (unsupported) on 5 consecutive days

Engraftment studies using EDTA-peripheral blood samples will be taken to measure the relative proportion of donor and recipient nucleated cells present in both whole blood and circulating T cells. These studies will be used to define chimerism status and graft rejection (see Section 4.5.2 below).

4.5.2 Definitions of mixed chimerism and graft rejection

Engraftment status (or chimerism) will be determined by FACS sorted peripheral blood cell populations taken on days +30, +60, +90 and, in the absence of DLI or progressive loss of donor chimerism, thereafter only on day +365. If DLI is given, chimerism will also be performed at days 28 and 60 after each DLI, and then each 3 months until one year post-HPCT. More frequent chimerism (at least 4 weekly) should be performed if there is progressive loss of donor T cell chimerism at any stage post-HPCT. Chimerism will be assessed using DNA fingerprinting (RFLP analysis) of nucleated cell populations in peripheral blood.

Mixed chimerism is defined as the detection of donor T cells (CD3+), as a proportion of the total T cell population, of $>1\%$ and $\leq 95\%$. Graft rejection is defined as the absence of detectable

peripheral blood donor T cells. Full donor chimerism ("engraftment") is defined as >95% peripheral blood donor T cells.

4.5.3 Donor lymphocyte infusions

DLI is indicated for patients with:

1. progressive loss of donor T cell chimerism (on successive engraftment studies) within the first 6 months post-HPCT
2. persistent <50% donor T cell chimerism (on successive engraftment studies) within the first 6 months post-HPCT
3. graft rejection
4. disease relapse / progression

Irrespective of the indications above, DLI is contraindicated in patients who have previously suffered \geq grade II acute GVHD, or extensive stage chronic GVHD.

In patients who are eligible for DLI, the following steps should be undertaken:

1. cease immunosuppression (CsA +/- steroids)
2. repeat engraftment studies 4 weeks after cessation of immunosuppression
3. in the absence of developing **a.** progressive T cell chimerism, and / or **b.** significant (\geq grade II) GVHD, and / or **c.** significant disease response after cessation of immunosuppression, then proceed with 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 GVHD (grade II-IV).

Initially 3×10^6 CD3+ cells/kg recipient body weight will be infused, progressing sequentially to 1×10^7 CD3+ cells/kg, then 3×10^7 CD3+ cells/kg, then 1×10^8 CD3+ cells/kg recipient body weight.

Note that for patients with progressive disease despite full donor chimerism, at the Investigator's discretion, use of interferon to induce GVHD (and a GVM effect) is permitted prior to proceeding with DLI.

5. PHARMACEUTICAL INFORMATION

5.1 *Investigational agent*

TCZ is a humanized anti-IL-6R mAb which binds to both soluble and transmembrane IL-6R, inhibiting IL-6 binding, and thus blocking IL-6 signalling through both receptors. TCZ is specific for IL-6R and does not block signalling of other IL-6 family cytokines. Single dose pharmacokinetic studies in animals and humans have shown that clearance of TCZ is slow, with a half-life of 6 to >9 days.²⁸⁻³⁰

Age, sex and ethnicity do not affect the pharmacokinetics of TCZ. Although no formal studies have been carried out in patients with renal or hepatic impairment, available data suggest that mild renal impairment (estimated creatinine clearance between 50 and 80 ml/min) does not impact the pharmacokinetics of TCZ. Furthermore, TCZ clearance is not affected by concomitant use of MTX or corticosteroids.²⁹

TCZ may reverse the reduced expression of hepatic CYP isozymes associated with chronic inflammation in patients with RA and other systemic inflammatory disorders, potentially including HPCT. Theoretically, this could result in increased serum concentrations of drugs metabolized via the CYP450 enzyme pathway, including atorvastatin, calcium channel blockers, tacrolimus and cyclosporine. Thus, monitoring of drug levels and / or dosage adjustments of these drugs is required when using TCZ. This will occur routinely in patients enrolled in this study (see Section 6 – Study Calendar). As detailed in Section 4.4.1, cyclosporine levels will be routinely performed at least twice per week in the first 100 days post-transplant, with cyclosporine dose adjusted to maintain trough levels at 140-300 ng/ml as part of standard supportive care in this study.^{29,30}

5.2 *Administration*

TCZ will be administered on an inpatient basis, as a single dose on day -1 of conditioning (day-1). TCZ is administered as an IV infusion over 60mins using a standard infusion set. TCZ is administered at room temperature. Dose is 8mg/kg, up to a maximum dose of 800mg. Due to a lack of physical or biocompatibility studies, TCZ will not be infused with other drugs in the same intravenous line.

5.3 *Toxicities*

Almost all available safety data of TCZ derive from randomized controlled clinical trials and their uncontrolled open-label extensions in refractory RA. These randomized phase III studies compared TCZ as either monotherapy or combination therapy with DMARDs (typically methotrexate) to placebo as treatment for refractory arthritis.

Adverse events attributable to TCZ from these studies included.²⁸⁻³⁰

Common toxicities (incidence \geq 1:100)

a. Infusion reactions

Infusion reactions, defined as selected events occurring during or within 24hrs of infusion, occur in 7-15% of patients. These events are generally transient and mild in

severity and are not treatment limiting. These reactions include skin reactions (pruritis, rash and urticaria), headache and hypertension (primarily during infusion).

Standard pre-medication with paracetamol 1gm PO and phenergan (promethazine) 12.5mg IV will be administered 15-30mins pre-TCZ infusion to prevent TCZ-related infusion reactions (see Section 4.1).

b. Infections

Rates of serious infection with repeated exposure to TCZ in the treatment of RA are 3.4-5.3 events / 100 patient years (PY). The most commonly associated serious infections are pneumonia (0.7 events / 100 PY) and cellulitis (0.5 events / 100 PY). Other reported infections associated with TCZ therapy include Herpes zoster reactivation, diverticulitis and cases of opportunistic infection (0.3 events / 100 PY).

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.

c. Dermatological

Skin rashes, pruritis and urticaria have been described during TCZ infusion (see "Infusion reactions" above).

d. Gastrointestinal

Diarrhoea, abdominal pain and gastritis occurred in 1-5% of patients in the randomized RA studies. GI side effects occurred at a similar frequency in placebo-treated patients on these studies.

e. Liver function abnormalities

The incidence of elevations in liver transaminases (ALT / AST) $>3 \times$ ULN was 2.1% for patients treated with TCZ as monotherapy (compared to 4.9% of patients on MTX alone), and in 6.5% of patients treated with TCZ plus a DMARD (compared to 1.5% of patients on placebo + DMARD). Thus, the frequency of elevations of AST / ALT is increased when TCZ is used in combination with other hepatotoxic drugs, including MTX.

Most elevations in AST / ALT $>3 \times$ ULN were transient and resolved either spontaneously or after dose reduction and / or interruption of subsequent TCZ infusions and / or MTX. No clinically apparent hepatitis or hepatic impairment was observed in association with elevation of AST / ALT.

Patients with pre-existing AST / ALT levels $>3 \times$ ULN (i.e. $>$ pre-existing grade 1 toxicity) will be excluded from this trial (see Section 3.2). In addition, all patients will receive routine URSO as VOD prophylaxis (see Section 4.4.2).

Interim toxicity analysis will also be performed after each cohort of 10 patients has reached the day +100 follow-up mark. If significant liver toxicity (defined as > grade 2 toxicity in $\geq 20\%$ of recipients unrelated to GVHD) is experienced, MMF will be substituted for MTX as primary GVHD prophylaxis. If significant liver toxicity persists in subsequent cohorts of (10) patients despite MMF substitution, the trial will be stopped (see Section 8.4).

f. Altered lipid profile

Changes in lipid profile is commonly observed in patients treated with repeated TCZ infusions, with ~24% of patients experiencing sustained elevations in total cholesterol >6.2 mmol/l.

Increases in triglyceride levels are much less frequent and have not been associated with any symptoms and / or toxicity, including pancreatitis.

As per Institutional guidelines, all patients in this study will have their lipid profiles determined pre-transplantation and again at day +100 post-transplant. If sustained elevation in serum cholesterol is experienced beyond day +100, patients will be commenced on lipid lowering therapy.

g. Haematological

TCZ is associated with transient decreases in neutrophil counts via inhibition of the biological effect of IL-6 on the recruitment of neutrophils into peripheral blood. The incidence of moderate – severe neutropenia (neutrophil count $<1.0 \times 10^9/L$) is 3.4%, and 0.3% for severe neutropenia (neutrophil count $<0.5 \times 10^9/L$).

Thrombocytopenia (platelet count $<100 \times 10^9/L$) has also been reported to occur in 1.7% of patients.

Given that grade 3-4 neutropenia and thrombocytopenia is a normal and expected result of the HPCT conditioning regimen in all patients on this trial, assessment of TCZ haematological toxicity on the trial will be via assessment of engraftment outcomes. Interim toxicity analysis will also be performed after each cohort of 10 patients has reached the day +100 follow-up mark. If unacceptable delay in engraftment occurs (as defined by median neutrophil engraftment > 25 days despite G-CSF and / or median platelet engraftment > 25 days), the trial will be stopped (see Section 8.4).

h. Other

Immunogenicity. Development of anti-TCZ antibodies occurs in 1.6% of patients, with neutralizing antibodies seen in 1.1%. As patients will only receive a single TCZ infusion in this study, anti-TCZ anti-bodies will not be screened for.

2. Uncommon toxicities (incidence <1:100)

a. Hypersensitivity reactions (including anaphylaxis)

Clinically significant hypersensitivity reactions and / or anaphylaxis during TCZ infusion are uncommon (incidence 0.2-0.5%) and are generally observed during the 2nd to 5th infusions of TCZ in refractory RA. Patients in this study will receive a single infusion of TCZ only at day -1 of HPCT (see Section 4.1).

b. Gastrointestinal

Non-fatal GI perforation occurred in 0.65-0.8% of patients enrolled in the randomized studies. Most cases involved the lower GIT and appeared primarily as complications of diverticulitis. Overall, the rate of GI perforation in patients treated with TCZ is estimated at 2.6-2.8 events / 1000 PY. In comparison, the GI perforation rate in RA patients treated with corticosteroids is 3.9 events / 1000 PY.

Patients with a past history of complicated diverticulitis (fistulae, abscess formation or perforation) are excluded from this study.

c. Liver function abnormalities

Increases in bilirubin levels occurs rarely in TCZ-treated patients (incidence of elevations >3 x ULN 0.5%) and appear unrelated to elevations in AST / ALT.

Patients with pre-existing bilirubin >30µmol/L will be excluded from this trial. In addition, all patients will receive routine URSO as VOD prophylaxis (see Section 4.4.2).

Interim toxicity analysis will also be performed after each cohort of 10 patients has passed the day +100 follow-up mark. If significant liver toxicity (defined as > grade 2 toxicity in ≥ 20% of recipients unrelated to GVHD) is experienced, MMF will be substituted for MTX as primary GVHD prophylaxis. If significant liver toxicity persists in subsequent cohorts of (10) patients despite MMF substitution, the trial will be stopped (see Section 8.4).

In the published case report and case series of TCZ as treatment of steroid-refractory GVHD, no unexpected toxicities were observed. In the case series, 6 patients were treated with TCZ 8mg/kg every 3-4 weeks for a total of 31 infusions. Grade 1-2 elevations in liver transaminases were seen after 8 of 31 infusions (26%) and treatment was discontinued in one patient due to worsening of pre-existing grade 1 hyperbilirubinaemia.

6. STUDY CALENDAR

Evaluations to be conducted include:

6.1 *Pre-HPCT evaluations*

6.1.1 *Routine evaluations* (as per Institutional guidelines)

- Medical assessment: including relevant medical history, physical examination, ECOG, vital signs, weight.
- Assessment of specific organ function: gated heart pool scan or echocardiogram; chest x-ray; respiratory function testing; liver function tests; estimated GFR
- Full re-staging of underlying disease
- Other blood investigations: FBE; coagulation profile; HIV / HBC / HCV / CMV / EBV / VZV / HSV I + II / toxoplasmosis / syphilis serology; pregnancy testing (females only)
- Fasting lipid profile including serum cholesterol and triglyceride levels
- Dermatology / dental / social work +/- gynaecology (for female patients only) review

6.1.2 *Study-specific evaluations / procedures*

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

6.2 *During / after HPCT*

For study purposes, patients will be medically assessed weekly from the start of the conditioning regimen until day +100, 3 weekly from day +100 to day +180, and monthly from day +180 to 1 year post HPCT and at 2 years post HPCT. 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 +100 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 will be performed at day +100 post-HPCT. CsA monitoring, CMV monitoring (qPCR) and other routine post-transplant assessments will be performed as per Institutional guidelines (see Supportive Care Guidelines above).

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. If feasible,

sections of these biopsies may also be assessed for IL-6R expression and / or other cytokine / activation signals by immunohistochemistry.

Study-specific evaluations required include:

1. Reassessment of remission status of underlying disease at 3, 6, 12 and 24 months post-HPCT.
2. Engraftment studies at 1, 2, 3, and 12 months post-HPCT.
3. Study-specific assays to be undertaken by QIMR:
 - Measurement of plasma and cell derived cytokine levels including IL-6/IL-6R (by both immunoassay and bioassay) prior to commencement of conditioning and at days 0, +3, +7, +14, +21, +30, +60, +90, +180, +360. These assays require 22 mls of blood collected into heparin tubes.
 - Numbers 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.
 - Measurement of TCZ levels (by ELISA) may be undertaken at days +3, +7, +14, +21 and +30. This requires 8 mls of blood collected into a clotted tube. These samples will be processed at QIMR and sent to ROCHE (The Netherlands) for analysis.

Study Schema

	Pre-HPCT	Conditioning	D0 – D30 (± 3 day)	D30 – D100 (± 3 days)	D100 – D180 (± 5 days)	6 - 12months (± 2 weeks)	24 months (± 1 month)
Medical Assessment* ##	X	X (weekly)	X (weekly)	X (weekly)	X (3 rd weekly)	X (monthly)	X
ECOG*	X	X (weekly)	X (weekly)	X (weekly)	X (3 rd weekly)	X (monthly)	X
Vital signs*	X	X (weekly)	X (weekly)	X (weekly)	X (3 rd weekly)	X (monthly)	X
Weight*	X	X (weekly)	X (weekly)	X (weekly)	X (3 rd weekly)	X (monthly)	X
Routine pre-HPCT investigations / referrals*	X						
Disease restaging	X			X (3 months)	X (6months)	X (12months)	X
Written Informed Consent	X						
Review Inclusion/ Exclusion Criteria	X						
TCZ Administration		X (D-1)					
Adverse Events		X (D-1)	X	X			
GVHD scoring			X (weekly)	X (weekly)	X (3 rd weekly)	X (monthly)	X
Engraftment studies				X (D+30, D+60, D+90)		X (12months)	
TCZ levels ^{&}			X (D+3, D+7, D+14, D+21, D+30)				
Serum IL-6/IL-6R levels and T cell subsets ^{\$\$}		X (prior to commencement of conditioning)	X (D0, D+3, D+7 D+14, D+21, D+30)	X (D+60, D+90)	X (D+180)	X (D+360)	

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*Patients will be medically assessed prior to commencing conditioning, then weekly from the start of the conditioning regimen until day +100, 3rd weekly from day +100 to day +180, and monthly from day +180 to 1 year post HPCT and at 2 years post HPCT. Medical assessment will include physical examination, ECOG, vital signs, weight, and after commencing conditioning, toxicity assessment including GVHD assessment and assessment of adverse events (from day -1 to day +100).

##FBE and ELFT will be performed at each medical review. Fasting lipid profile including serum cholesterol and triglyceride levels will be performed pre-HPCT and at day +100 post-HPCT. CsA monitoring, CMV monitoring (qPCR) and other routine post-transplant assessments will be performed as per Institutional guidelines.

& TCZ levels may be measured at days +3, +7, +14, +21 and +30. These assays require 8 mls of blood collected into a clotted tube. These samples will be processed by QIMR and sent on to ROCHE (The Netherlands) for analysis.

\$\$ Serum IL-6/IL-6R levels will be measured prior to commencement of conditioning pre-HPCT, then at days 0, +3, +7, +14, +21, +30, +60, +90, +180 and +360. These assays require 22 mls of blood collected into heparin tubes. 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. These samples will be processed and analysed at QIMR.

7. MEASUREMENT OF EFFECT

7.1 Endpoints

7.1.1 Primary endpoint

The primary endpoint is incidence of grade II-IV (moderate – severe) acute GVHD at day +100.

7.1.2 Secondary endpoints

7.1.2.1 IL-6/IL-6R and TCZ levels post-HPCT (see Section 6.2)

7.1.2.2 Effector and regulatory T cell subsets post-HPCT (see Section 6.2).

7.1.2.3 Incidence of engraftment

7.1.2.4 Infection rate

7.1.2.5 Incidence of liver toxicity

7.1.2.6 Incidence of chronic GVHD at 2 years

7.1.2.7 Transplant related mortality (TRM)

7.1.2.8 Progression free survival (PFS)

7.1.2.9 Overall survival (OS)

7.2 Definition of endpoints

7.2.1 Acute graft versus host disease (GVHD)

Acute GVHD is defined as GVHD occurring prior to day +100. Acute GVHD will be assessed and graded according to the Seattle criteria (see Tables below).³³ Whenever possible, histological biopsies will be taken to confirm diagnosis of GVHD. If feasible, sections of these biopsies may also be sent for assessment of IL-6R expression and / or other cytokine / activation signals.

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
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				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

7.2.2 Plasma and cell derived cytokine levels (including IL-6/IL-6R) will be measured by both immunoassay and bioassay prior to commencement of conditioning, and at days 0, +3, +7, +14, +21, +30, +60, +90, +180 and +360 post-HPCT. TCZ levels may be determined at days +3, +7, +14, +21 and +30 post-HPCT.

7.2.3 Numbers of effector and regulatory T cell subsets will be determined prior to commencement of conditioning, and at days 0, +3, +7, +14, +21, +30, +60, +90, +180 and +360 post-HPCT by flow cytometry on peripheral blood.

7.2.4 *Engraftment*

Engraftment criteria are as defined in Section 4. The kinetics of T cell and myeloid chimerism will be studied by graphing the percentage of donor T cells and percentage of donor myeloid cells as a function of time since transplant for each eligible patient. Incidence of graft rejection and requirement for DLI will also be recorded

7.2.5 *Infection rate*

All infectious complications will be recorded, including microbiological diagnoses. Incidence of CMV reactivation and / or disease will also be recorded.

7.2.6 *Liver toxicity*

Electrolyte and liver function testing will be performed at each medical review (see Section 4). All abnormal liver function testing will be graded as per CTCAE criteria, version 4 (see Appendix C).

Incidence of VOD will also be recorded. Diagnosis and treatment of VOD will be as per Institutional guidelines.

7.2.7 *Chronic GVHD*

Chronic GVHD is defined as GVHD occurring beyond day +100.

Chronic GVHD will be assessed and graded according to the Seattle criteria (see Table below).³⁴ Whenever possible, histological biopsies will be taken to confirm diagnosis of GVHD. If feasible, sections of these biopsies may also be assessed for IL-6R expression and / or other cytokine / activation signals by immunohistochemistry.

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

7.2.8 Transplant related mortality (TRM)

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

7.2.9 Progression free survival (PFS)

PFS is measured from commencement of TCZ at day -1 until relapse and / or progression of underlying disease. As patients are not assessed with respect to disease response until 3 months post-HPCT, only patients surviving >90 days post transplantation will be included in analysis of PFS

7.2.10 Overall survival (OS)

OS is measured from commencement of TCZ at day -1 until death from any cause.

7.3 Adverse events (AEs)

7.3.1 Definition

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 (TCZ), from day -1 to day +100.

Due to the expected toxicity of HPCT, only \geq grade 3 toxicities, or \geq grade 2 for infections, will be recorded as AEs.

Since all patients will develop grade 4 haematological toxicity due to the HPCT itself, this will not be reported as AEs / SAEs. Haematological toxicity will be specifically captured within the engraftment endpoints.

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 grade II-IV (moderate – severe) acute GVHD at day +100 post allogeneic HPCT.

Analysis of a historical allogeneic HPCT cohort at RBWH demonstrates that the incidence of grade II-IV acute GVHD is approximately 60%. To detect a 33% reduction in incidence of grade II-IV acute GVHD with 80% power and a two-sided significance level of 5%, a sample size of 48 patients is required.

8.2 Accrual rate

Based on the number of allogeneic HPCT performed at RBWH (approximately 80 per year), it is expected to recruit 32 patients per year, with enrolment completed within 18 months. Patients who withdraw or are withdrawn from study prior to commencement of conditioning or Tocilizumab administration will be replaced on the trial 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 100 day follow-up mark.

1. Unacceptable mortality or absence of efficacy. The primary endpoint is the incidence of grade II-IV acute GVHD. The expected incidence of day +100 mortality in this cohort is 25%. The expected grade II-IV acute GVHD in the target population using G-CSF mobilised HPC is 60% and for grade III-IV is 25%. The study will be closed at the interim time-point if the lower confidence interval for the incidence of mortality exceeds 25% or either grade II-IV acute GVHD exceeds 60% or grade III-IV acute GVHD exceeds 25%.
2. Unacceptable delay in engraftment (median neutrophil engraftment > 25 days despite G-CSF and/or median platelet engraftment > 25 days). Note that median times to neutrophil and platelet engraftment currently are 18 and 14 days.²
3. Liver toxicity (> grade 2 toxicity as per CTCAE v. 4 in ≥ 20% of recipients within the first 40 days post transplant unrelated to known GVHD, infection or other clearly defined drug effects). In this setting the GVHD prophylaxis will first revert to CsA and MMF. The study will stop if liver toxicity persists in the absence of MTX.

8.5 Independent External Review

Prof David Gottlieb (BMT Unit, Westmead Hospital, Sydney) 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 has passed the 100 day follow-up mark.

KEY REFERENCES

1. Tawara, I., *et al.* Interleukin-6 modulates graft-versus-host responses after experimental allogeneic bone marrow transplantation. *Clin Cancer Res* 17, 77-88 (2011).
2. Hill, G.R., *et al.* Allogeneic Stem Cell Transplantation with Peripheral Blood Stem Cells Mobilized by Pegylated G-CSF. *Biol Blood Marrow Transplant* 12, 603-607 (2006).
3. Deeg, H.J. How I treat refractory acute GVHD. *Blood* 109, 4119-4126 (2007).
4. Levine, J.E., *et al.* Graft-versus-host disease treatment: predictors of survival. *Biol Blood Marrow Transplant* 16, 1693-1699 (2010).
5. Kennedy, G.A., *et al.* Combination anti-thymocyte globulin and soluble TNF inhibitor +/- mycophenolate mofetil for treatment of steroid refractory acute GVHD. *Bone Marrow Transplant* 37, 1143-1147 (2006).
6. Ratanatharathorn, V., *et al.* Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood* 92, 2303-2314 (1998).
7. Nash, R.A., *et al.* Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood* 96, 2062-2068 (2000).
8. Bolwell, B., *et al.* A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. *Bone Marrow Transplant* 34, 621-625 (2004).
9. Nash, R.A., *et al.* A phase I/II study of mycophenolate mofetil in combination with cyclosporine for prophylaxis of acute graft-versus-host disease after myeloablative conditioning and allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 11, 495-505 (2005).
10. Yu, C., Seidel, K., Fitzsimmons, W.E., Sale, G. & Storb, R. Glucocorticoids fail to enhance the effect of FK506 and methotrexate in prevention of graft-versus-host disease after DLA-nonidentical, unrelated marrow transplantation. *Bone Marrow Transplant* 20, 137-141 (1997).
11. Ruutu, T., Volin, L., Parkkali, T., Juvonen, E. & Elonen, E. Cyclosporine, methotrexate, and methylprednisolone compared with cyclosporine and methotrexate for the prevention of graft-versus-host disease in bone marrow transplantation from HLA-identical sibling donor: a prospective randomized study. *Blood* 96, 2391-2398 (2000).
12. Antin, J.H., *et al.* Sirolimus, tacrolimus, and low-dose methotrexate for graft-versus-host disease prophylaxis in mismatched related donor or unrelated donor transplantation. *Blood* 102, 1601-1605 (2003).
13. Bacigalupo, A., *et al.* Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood* 98, 2942-2947 (2001).
14. Furlong, T., *et al.* Sirolimus in combination with cyclosporine or tacrolimus plus methotrexate for prevention of graft-versus-host disease following hematopoietic cell transplantation from unrelated donors. *Biol Blood Marrow Transplant* 14, 531-537 (2008).
15. Finke, J., *et al.* Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol* 10, 855-864 (2009).
16. Morris, E.S. & Hill, G.R. Advances in the understanding of acute graft-versus-host disease. *Br J Haematol* 137, 3-19 (2007).
17. Hill, G.R., *et al.* Interleukin-11 promotes T cell polarization and prevents acute graft-versus-host disease after allogeneic bone marrow transplantation. *J Clin Invest* 102, 115-123 (1998).
18. Hill, G.R. & Ferrara, J.L. The primacy of the gastrointestinal tract as a target organ of acute graft-versus-host disease: rationale for the use of cytokine shields in allogeneic bone marrow transplantation. *Blood* 95, 2754-2759 (2000).
19. Morris, E.S., MacDonald, K.P. & Hill, G.R. Stem cell mobilization with G-CSF analogs: a rational approach to separate GVHD and GVL? *Blood* 107, 3430-3435 (2006).
20. Chen, X., *et al.* Blockade of interleukin-6 signaling augments regulatory T-cell reconstitution and attenuates the severity of graft-versus-host disease. *Blood* 114, 891-900 (2009).
21. Markey, K.A., *et al.* Soluble lymphotoxin is an important effector molecule in GVHD and GVL. *Blood* 115, 122-132.

22. Scheller, J., Chalaris, A., Schmidt-Arras, D. & Rose-John, S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta* 1813, 878-888 (2011).
23. Febbraio, M.A., Rose-John, S. & Pedersen, B.K. Is interleukin-6 receptor blockade the Holy Grail for inflammatory diseases? *Clin Pharmacol Ther* 87, 396-398 (2010).
24. Symington, F.W., *et al.* The relationship of serum IL-6 levels to acute graft-versus-host disease and hepatorenal disease after human bone marrow transplantation. *Transplant.* 54, 457-462 (1992).
25. Schwaighofer, H., *et al.* Serum levels of interleukin 6, interleukin 8, and C-reactive protein after human allogeneic bone marrow transplantation. *Transplant.* 58, 430-436 (1994).
26. Gergis, U., *et al.* Effectiveness and safety of tocilizumab, an anti-interleukin-6 receptor monoclonal antibody, in a patient with refractory GI graft-versus-host disease. *J Clin Oncol* 28, e602-604 (2010).
27. Drobyski, W.R., *et al.* Tocilizumab for the treatment of steroid refractory graft versus host disease. *Biol Blood Marrow Transplant* (2011).
28. Singh, J.A., Beg, S. & Lopez-Olivo, M.A. Tocilizumab for rheumatoid arthritis: a Cochrane systematic review. *J Rheumatol* 38, 10-20 (2011).
29. Bannwarth, B. & Richez, C. Clinical safety of tocilizumab in rheumatoid arthritis. *Expert Opin Drug Saf* 10, 123-131 (2011).
30. Roche. Actemra: Product information. *Product information Roche, De Why, NSW 2099*(2011).
31. Ito, H., *et al.* A pilot randomized trial of a human anti-interleukin-6 receptor monoclonal antibody in active Crohn's disease. *Gastroenterology* 126, 989-996; discussion 947 (2004).
32. Matsuyama, M., *et al.* Anti-interleukin-6 receptor antibody (tocilizumab) treatment of multicentric Castleman's disease. *Intern Med* 46, 771-774 (2007).
33. Glucksberg, H., *et al.* Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation* 18, 295-304 (1974).
34. Shulman, H.M., *et al.* Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 69, 204-217 (1980).

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

APPENDIX B

Haematopoietic progenitor cell mobilization

G-CSF mobilized peripheral blood HPC will be used as the stem cell source.

Stem cell mobilization will be undertaken as per Institutional guidelines. Stem cell donors will receive G-CSF at 10µg/kg/day SC for 4 to 5 days, with peripheral blood stem cell collection performed via apheresis on day 5 +/- 6 if required. Donors will commence G-CSF on day -6 of the recipient's HPCT conditioning regimen.

Treatment Schema for Donor

Days	1	2	3	4	5	6
G-CSF 10 mcg/kg/SC	X	X	X	X	X	
HPC collection					X	X (if needed)

Peripheral blood stem cells will be mobilized on an apheresis machine via standard procedures. Donors will only commence apheresis if circulating peripheral blood (CD34 positive) stem cells are $>10 \times 10^6/L$ on the morning of planned collection. If $\geq 3 \times 10^6$ CD34 positive cells / kg recipient body weight is collected on the first day of apheresis, no further collections will occur. If $< 3 \times 10^6$ CD34 positive cells / kg recipient body weight is collected on the first day of apheresis, a second collection is permitted the following day. A third day of apheresis is not allowed.

There is no upper collection limit of CD34 stem cells. However, due to an association between CD34 count and risk of GVHD, no more than 8×10^6 CD34 positive cells / kg recipient body weight will be re-infused for sibling grafts, or 6×10^6 CD34 positive cells / kg recipient body weight re-infused for unrelated donor grafts (as per Institutional guidelines). Any excess CD34 cells collected above these values will be cryopreserved.

Donors who fail to mobilize sufficient peripheral CD34 positive cells to support engraftment may subsequently undergo bone marrow harvest under general anaesthesia on day 7 of G-CSF. In this circumstance, 15-20ml of marrow / kg recipient body-weight will be collected.

APPENDIX C

CTCAE version 4.0 grading scale for selected toxicities

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
	(none)	(mild)	(moderate)	(severe)	(life threatening/ debilitating)	Death
Infection						
Febrile Neutropenia	Nil	N/A	N/A	Present	Life threatening consequences / urgent intervention indicated	Death
Allergies						
Allergic reaction	Nil	Transient flushing or rash, drug fever <38°C; intervention not indicated	Intervention or infusion interruption indicated; responds promptly to symptomatic treatment; prophylactic medications indicated for <24 hrs	Prolonged; recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae	Life threatening consequences / urgent intervention indicated	Death
Rash (Drug related)	Nil	Macules / papules covering <10% BSA with or without symptoms	Macules / papules covering 10 - 30% BSA with or without symptoms	Macules / papules covering >30% BSA with or without associated symptoms; limiting self care ADL	N/A	N/A
Gastrointestinal						
Bilirubin	≤ ULN	>ULN -1.5xULN	>1.5 - 3.0 x ULN	>3.0 - 10 x ULN	>10 x ULN	Death
GGT	≤ ULN	>ULN -2.5xULN	>2.5 – 5.0 x ULN	>5.0 - 20 x ULN	>20 x ULN	Death
Alkaline Phosphatase	≤ ULN	>ULN -2.5xULN	>2.5 – 5.0 x ULN	>5.0 - 20 x ULN	>20 x ULN	Death
AST / ALT	≤ ULN	>ULN -3.0xULN	>3.0 – 5.0 x ULN	>5.0 - 20 x ULN	>20 x ULN	Death
Oral	None	Asymptomatic / mild (soreness / erythema) – no intervention required	Moderate pain not interfering with oral intake / modified diet required	Severe pain interfering with oral intake	Life threatening consequences / urgent intervention indicated	Death
Nausea	None	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral, caloric or fluid intake; enteral feeding, TPN or hospitalization indicated	N/A	N/A

ULN = upper limit of normal