Addition of interleukin-6 inhibition with tocilizumab to standard graft-versus-host disease prophylaxis after allogeneic stem-cell transplantation: a phase 1/2 trial

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Summary

Background Interleukin 6 mediates graft-versus-host disease (GVHD) in experimental allogeneic stem-cell transplantation (allogeneic SCT) and represents an attractive therapeutic target. We aimed to assess whether the humanised anti-interleukin-6 receptor monoclonal antibody, tocilizumab, could attenuate the incidence of acute GVHD.

Methods We undertook a single-group, single-institution phase 1/2 study at the Royal Brisbane and Women's Hospital Bone Marrow Transplantation unit, QLD, Australia. Eligible patients were 18–65 years old and underwent T-replete HLA-matched allogeneic SCT with either total body irradiation-based myeloablative or reduced-intensity conditioning from unrelated or sibling donors. One intravenous dose of tocilizumab (8 mg/kg, capped at 800 mg, over 60 mins' infusion) was given the day before allogeneic SCT along with standard GVHD prophylaxis (cyclosporin [5 mg/kg per day on days –1 to +1, then 3 mg/kg per day to maintain therapeutic levels (trough levels of 140–300 ng/mL) for 100 days plus methotrexate [15 mg/m² on day 1, then 10 mg/m² on days 3, 6, and 11]). The primary endpoint was incidence of grade 2–4 acute GVHD at day 100, assessed and graded as per the Seattle criteria. Immunological profiles were compared with a non-randomised group of patients receiving allogeneic SCT, but not treated with tocilizumab. This trial is registered with the Australian and New Zealand Clinical Trials Registry, number ACTRN12612000726853.

Findings Between Jan 19, 2012, and Aug 27, 2013, 48 eligible patients receiving cyclosporin and methotrexate as GVHD prophylaxis were enrolled into the study. The incidence of grade 2–4 acute GVHD in patients treated with tocilizumab at day 100 was 12% (95% CI 5–24), and the incidence of grade 3–4 acute GVHD was 4% (1–13). Grade 2–4 acute GVHD involving the skin developed in five (10%) patients of 48 treated with tocilizumab, involving the gastrointestinal tract in four (8%) patients; there were no reported cases involving the liver. Low incidences of grade 2–4 acute GVHD were noted in patients receiving both myeloablative total body irradiation-based conditioning (12% [95% CI 2–34) and fludarabine and melphalan reduced-intensity conditioning (12% [4–27]). Immune reconstitution was preserved in recipients of interleukin-6 receptor inhibition, but qualitatively modified with suppression of known pathogenic STAT3-dependent pathways.

Interpretation Interleukin 6 is the main detectable and dysregulated cytokine secreted after allogeneic SCT and its inhibition is a potential new and simple strategy to protect from acute GVHD despite robust immune reconstitution; a randomised, controlled trial assessing tocilizumab in addition to standard GVHD prophylaxis in these patients is warranted.

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Introduction

Allogeneic stem-cell transplantation (SCT) remains the curative therapy for a range of malignant conditions. Allogeneic SCT acts by mediating immunological graft-versus-leukaemia effects directed against residual leukaemia after transplantation. Unfortunately, these immune responses can also be directed towards normal host organs and tissues, resulting in acute graft-versus-host disease (GVHD) in the skin, gut, and liver, which causes substantial mortality and morbidity.

Pro-inflammatory cytokines have been recognised as mediators of acute GVHD in experimental systems, particularly interleukin 1, tumour necrosis factor (TNF), interferon γ (IFNγ), and interleukin 6.[1] These cytokines are generated by both conditioning-induced tissue damage and donor T-cells, the latter perpetuating GVHD via direct cytotoxic effects on host tissue and the promotion of pathogenic alloantigen-specific Th1 and Th17 differentiation.[1] To date, interleukin 1 and TNF have been extensively targeted in clinical allogeneic SCT with varying success.[2,3] Interleukin 6 has recently been proposed as a key mediator in the pathogenesis of GVHD in experimental systems.[4,5] Interleukin 6 can be produced by most cell types with T-cells and myeloid cells secreting large quantities.[6] Interleukin 6 signals via an interleukin-6 receptor/gp130 heterodimer and, while the interleukin-6 receptor has restricted tissue distribution (T cells, monocytes, neutrophils, and hepatocytes), the gp130

component is ubiquitously expressed. The interleukin-6 receptor is cleaved by metalloproteases into a soluble form that then binds interleukin 6 and signals in-trans in any tissue expressing gp130.[6] The in-trans signalling pathway is highly inflammatory, while classic signalling in cells directly expressing the interleukin-6 receptor is crucial for the differentiation of donor T-cells down a Th17 pathway, which is capable of mediating GVHD.[6,7] A humanised anti-interleukin-6 receptor monoclonal antibody, tocilizumab, has been extensively studied in humans; it inhibits both classical and trans-signalling, and is approved for clinical use for treatment of rheumatoid arthritis.[8]

For the **protocol** see http://www.qimrberghofer.edu.au/page/Lab/Bone_Marrow_Transplantation/Clinical_Trial_Protocols

No articles had been published that used interleukin-6 inhibition for prevention of GVHD in patients undergoing haemopoietic stem-cell transplantation when we planned this study or subsequently. However, we and others had published preclinical data suggesting that interleukin 6 was an important pathogeneic cytokine mediating GVHD.[4,5] We thus hypothesised that tocilizumab could attenuate the incidence of acute GVHD.

Methods

Study design and participants

In this prospective, single institution, single group phase 1/2 study, eligible patients were enrolled from the Royal Brisbane and Women's Hospital (RBWH BMT unit), Brisbane, QLD, Australia and were aged 18–65 years. Patients underwent T-replete HLA-matched allogeneic SCT with either myeloablative or reduced-intensity conditioning from matched-unrelated or matched-sibling donors. Eligible patients also had to have an Eastern Cooperative Oncology Group performance status of 2 or less, total bilirubin 30 µmol/L or less, aminotransferases 3·0×upper limit of normal or less, creatinine clearance 50 mL per min/1·73 m² or greater, left ventricular ejection fraction 40% or greater, and pulmonary diffusion capacity 40% predicted or greater. Unrelated donors were matched at both alleles of HLA–A, B, C, DRB1, and DQ loci with high-resolution sequence-based typing (10/10 match); sibling donors were matched at both alleles of HLA–A, B, and C loci with intermediate resolution sequence-specific primers, and at HLA-DRB1 loci with sequence-based typing methods (8/8 match). A control cohort of patients derived from consecutive matched participants in an observational study (RBWH ethics approval, code LR 09/10) also undertaken at our institution was used to examine the immunological consequences of interleukin-6 inhibition. This study examined the relationship between plasma cytokine levels and cellular immune function after allogeneic SCT at identical time points to those used in the tocilizumab trial. Recruitment to this study began in December 2009 and continued in parallel to the end of recruitment for the tocilizumab trial, with all included patients during this time receiving allogeneic SCT with myeloablative total body irradiation-based conditioning or fludarabine and melphalan reduced-intensity conditioning, but without the administration of tocilizumab. Patients were offered participation in either the observational or interventional tocilizumab phase 1/2 trial during this time period; participation was chosen by the patient.

See Online for appendix

The trial protocol was approved by both the QIMR Berghofer Medical Research Institute and RBWH Human Research Ethics Committees (HREC/11/QRBW/345) with written informed consent obtained from all patients.

Procedures

Patients receiving myeloablative conditioning were given cyclophosphamide (60 mg/kg per day for days –5 and –4, where day 0 was day of allogeneic SCT plus total body irradiation (12 Gy total over days –3 to –1). Those receiving reduced-intensity conditioning were given fludarabine 25 mg/m² per day for days –7 to –3 plus melphalan 120 mg/m² given on day –2. Granulocyte-colony stimulating factor (G-CSF) mobilised peripheral blood stem cells (PBSC) were transplanted in all patients without anti-thymocyte globulin, campath-1H, or ex-vivo T-cell depletion. GVHD prophylaxis was cyclosporin (5 mg/kg per day on days –1 to +1, then 3 mg/kg per day to maintain therapeutic levels (trough levels of 140–300 ng/mL) for 100 days (with weaning thereafter at clinician discretion) plus methotrexate (15 mg/m² on day 1, then 10 mg/m² on days 3, 6, and 11).

One intravenous dose of tocilizumab (8 mg/kg, capped at 800 mg, over 60 mins' infusion) was given the day before allogeneic SCT. The timing of administration was based on preclinical data[5] and systemic interleukin-6 dysregulation in patients on the observational study (appendix p 5) that occurred only after conditioning and allogeneic SCT.

To confirm that interleukin-6 receptor monoclonal antibody blocked receptor signalling in vivo, we examined the expression of phosphorylated-STAT3 (pSTAT3) in CD14 monocytes, CD4 T-cells, and CD8 T-cells (where fresh cells were available) from patients both untreated and treated with tocilizumab at day 30. Exogenous recombinant interleukin 6 and G-CSF were used as positive controls to induce pSTAT3 expression. Chimerism was determined via short-tandem-repeat analysis of FACS-sorted populations in peripheral blood. We undertook non-hypothesis driven microarray-based RNA transcription profiling of monocytes (NCBI GEO accession number GSE61201) and hypothesis-driven MiSeq-based (Illumina, version 2) profiling in CD4 T-cells 30 days after allogeneic SCT, when interleukin 6 signalling was functionally inhibited as determined by pSTAT3 expression. The reasons for the two different experimental approaches reflect the fact that we hypothesised that interleukin-6 receptor inhibition would result in altered Th17 differentiation and the targeted MiSeq approach allowed us to focus our analysis on relevant transcription factors and cytokines with the highest level of sensitivity. By contrast, we had very little understanding of the

potential effects of interleukin-6 receptor inhibition on myeloid cells and thus used a non-hypothesis driven approach. We excluded patients with acute GVHD (grade 2–4) from the mRNA analysis at day 30 to avoid this being a confounding variable. Monocyte gene expression analysis was done with nucleic acid hybridisation to Illumina array technology (Human HT12v4 expression BeadChip) and scanned by an iScan Microarray Scanner (Illumina). Gene expression was determined using GenomeStudio (Illumina) and GeneSpring GX v12.5 (Agilent Technologies) software (appendix pp 3–4). Cytokine analyses including interleukin-6 concentrations were determined by immunoassay (appendix p 2).

Outcomes

The primary endpoint was incidence of moderate to severe (grade 2–4) acute GVHD at day 100, assessed and graded as per the Seattle criteria.[9] Secondary endpoints included time to engraftment (where neutrophil engraftment was defined as the first day of neutrophil count post nadir of greater than 0·5 cells×10^9 per L on 3 consecutive days, and platelet engraftment was the first day of platelet count post-nadir of greater than 20 cells×10^9 per L [unsupported] on 5 consecutive days, respectively), grade 2 or greater infection (as per the Common Terminology Criteria for Adverse Events criteria, version 4.03), grade 3 or greater liver toxicity (liver enzymes >5×upper limit of normal or bilirubin >3×upper limit of normal), chronic GVHD (as per the Seattle criteria),[9] transplant-related mortality, progression-free survival, and overall survival.

Statistical analysis

Analysis of historical T-replete allogeneic SCT cohorts showed an incidence of grade 2–4 acute GVHD of about 60%. To detect a 33% reduction in incidence of grade 2–4 acute GVHD (to <40%) with 80% power and a two-sided significance level of 5%, we calculated that a sample size of 48 patients was required. Survival analyses were done with Kaplan-Meier methods. Relapse and non-relapse mortality were competing risks for each other, while relapse and death were competing risks for GVHD. Competing risk regression was used to estimate cumulative incidence of GVHD and relapse (R software, version 2.15.3).[10] The non-parametric Mann-Whitney U test (Graphpad Prism version 6.05) was used for comparisons of two groups and the paired Wilcoxon-rank sum test corrected with the Benjamini & Hochberg method used for multiple comparisons (R software, version 2.15.3). $p<0·05$ was considered statistically significant. Array data were processed in GenomeStudio (Illumina) and analysed with Lumi[11] and BRB ArrayTools[12] as described previously.[13] Data were transformed by variance stabilisation transformation[14] and then normalised by robust spline normalisation.[15] Differentially expressed genes were identified by unpaired t-test with univariate permutation correction.[12] Additional methods can be found in the appendix (pp 2–4).

Role of the funding source

This was an investigator-driven and funded study without any involvement by a commercial sponsor. The corresponding author had full access to all of the data and the final responsibility to submit for publication. GAK, AV, PZ, JL, ES, JA, SKT, and GRH had access to the primary clinical data.

This trial is registered with the Australian and New Zealand Clinical Trials Registry, number ACTRN12612000726853.

Results

Between Jan 19, 2012, and Aug 27, 2013, 48 eligible patients receiving cyclosporin and methotrexate as GVHD prophylaxis were enrolled into the study. Two patients who did not receive GVHD prophylaxis with cyclosporin and methotrexate were major protocol violations and thus excluded and replaced. Table 1 shows

	Interleukin-6 receptor monoclonal antibody treated population (n=48)
Age (years)	48 (22–64)
Sex	
Men	30 (62%)
Women	18 (38%)
Woman donor to man recipient	10 (21%)
Disease	
Acute myeloid leukaemia	26 (54%)
Acute lymphoblastic leukaemia	10 (21%)
Myelodysplasia	5 (10%)
Lymphoproliferative disorder	5 (10%)
Myeloproliferative disorder	2 (4%)
Disease status	
Early	25 (52%)
Advanced	23 (48%)
Donor source	
Related PBSC	22 (46%)
Unrelated PBSC	26 (54%)
Conditioning	
Cy/TBI	16 (33%)
Flu/Mel	32 (67%)
Cytomegalovirus status	
Recipient+/donor+	17 (35%)
Recipient+/donor–	14 (29%)
Recipient–/donor+	7 (15%)
Recipient–/donor–	10 (21%)

Data are median (range) or number (%). Advanced disease defined as acute leukaemia beyond first complete remission, secondary leukaemia, and chronic myeloid leukaemia beyond first chronic phase. Lymphoproliferative disorders included chronic lymphocytic leukaemia, multiple myeloma, Hodgkin's disease, and non-Hodgkin's lymphoma. Myeloproliferative disorders included chronic myeloid leukaemia and myelofibrosis. PBSC=peripheral blood stem cells. Cy/TBI=cyclophosphamide/total body irradiation. Flu/Mel=fludarabine/melphalan.

Table 1: **Patient characteristics**

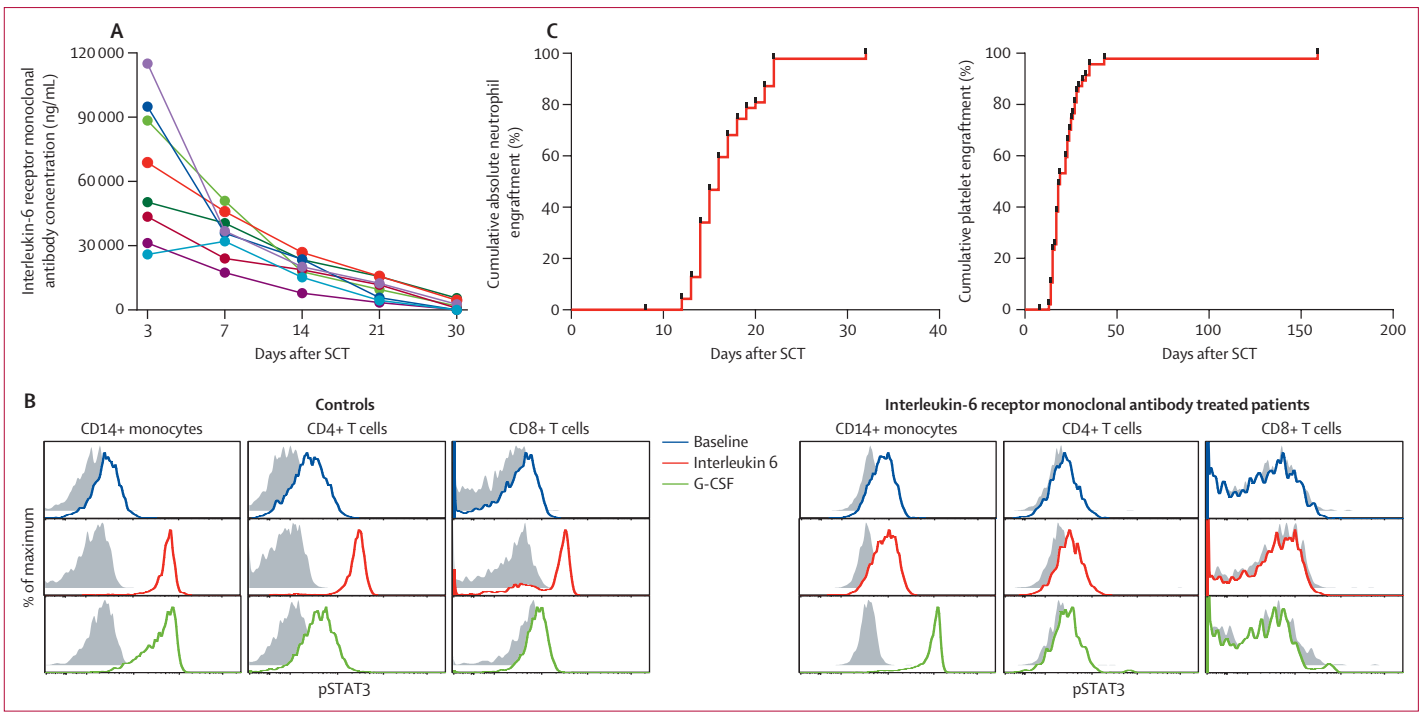

Figure 1: **Interleukin-6 receptor inhibition after allogeneic stem cell transplantation**
Interleukin-6 receptor monoclonal antibody concentration in sera of eight consecutive patients treated with tocilizumab up to 30 days after allogeneic SCT; each line represents one patient (A). Representative pSTAT3 expression in CD14 monocytes, CD4 T cells, and CD8 T cells in 26 untreated recipients compared with 11 patients treated with tocilizumab (B). Interleukin-6 and G-CSF recombinant proteins were used as positive and negative controls for interleukin-6 receptor stimulation. Note neither CD4 T cells nor CD8 T cells respond to G-CSF, consistent with the known low or absent expression of the G-CSF receptor on T-cells. Grey histograms represent isotype control stained cells. Quantitative data for each patient is shown in appendix p 5. Cumulative absolute neutrophil count and platelet engraftment in all 48 patients treated with tocilizumab over time (C).

patient characteristics. Enrolment is complete with a median follow-up of 497 days (IQR 310–622).

Cytokine dysregulation after allogeneic SCT was determined in a separate cohort of patients enrolled in an immune profiling study of recipients conditioned with myeloablative (n=27) or reduced-intensity conditioning (n=26) receiving standard GVHD prophylaxis without tocilizumab treatment. Interleukin 6 concentrations peaked at day 7 with a fall at day 14 and returned to baseline by day 30 (appendix p 5). Interleukin-6 dysregulation was equivalent in recipients of matched sibling or unrelated donor grafts (data not shown), but was proportional to the intensity of conditioning (appendix p 5).

Pharmacokinetic analysis of eight consecutive patients confirmed high concentrations of interleukin-6 receptor monoclonal antibodies at day 3 (mean 64·7 μg/mL), which persisted in all patients 3 weeks after allogeneic SCT (mean 9·8 μg/mL) and remained above the level of detection (100 ng/mL) in six (75%) patients at day 30 (mean 1·9 μg/mL; figure 1A). Soluble interleukin-6 receptor concentrations at day 30 correlated with residual antibody concentrations in serum (appendix p 5). Interleukin-6 receptor blockade in vivo prevented STAT3 phosphorylation in both monocytes and T-cells in response to interleukin 6 (figure 1B; appendix p 5). Monocytes from interleukin-6 receptor monoclonal antibody treated patients still phosphorylated STAT3 in response to G-CSF confirming the specificity of interleukin-6 blockade.

All 48 patients treated with tocilizumab achieved neutrophil engraftment at a median of 16 days (range 12–32), and 47 (98%) achieved platelet engraftment at 18 days (range 13–43). Time to engraftment in patients given interleukin-6 receptor monoclonal antibodies appeared similar to our own historical and published data (figure 1C).[16,17] 39 (87%) of 45 assessable patients achieved full donor T-cell chimerism and six were mixed T-cell chimeric with 81–87% donor CD3 T cells by day 30. By day 90, 44 (94%) of 47 surviving patients had achieved full donor T-cell chimerism; by 6–12 months all 40 surviving patients in remission were fully donor T-cell chimeric. No graft rejection was observed.

The incidence of grade 2–4 acute GVHD in patients treated with tocilizumab at day 100 was 12% (95% CI 5–24; figure 2A), and the incidence of grade 3–4 acute GVHD was 4% (1–13; figure 2B). Grade 2–4 acute GVHD involving the skin developed in five (10%) patients of 48 treated with tocilizumab, involving the gastrointestinal tract in four (8%) patients; there were no reported cases involving the liver. Low incidences of grade 2–4 acute GVHD were noted in patients receiving both

myeloablative total body irradiation-based conditioning (12% [95% CI 2–34]; appendix p 6) and fludarabine and melphalan reduced-intensity conditioning (12% [4–27]; appendix p 6).

With a median follow-up of 497 days (IQR 310–622), the cumulative rate of relapse was 27% (95% CI 15–41; figure 2C). With a median follow-up of 474 days (IQR 233–581), 51% (95% CI 35–65) of individuals developed chronic GVHD (figure 2D) and 22% (11–36) developed extensive stage chronic GVHD (figure 2E). Overall transplant-related mortality in patients receiving tocilizumab was 4%, comprising two non-relapse related deaths occurring on-study due to complications of MRSA infection (on day 30) and chronic liver GVHD (day 300), respectively. Overall survival at 24 months was 84% (95% CI 74–96; figure 2F) and overall progression-free survival at 24 months was 68% (95% CI 56–84; appendix p 6).

No significant tocilizumab-related infusion reactions were noted. 20 episodes of grade 3 liver toxicity were noted in 12 (25%) of 48 treated patients before day 40 (the period of tocilizumab antibody exposure): one (5%) was possibly related to tocilizumab, other toxicities occurred in patients who had sepsis (five [25%]), or azole administration (14 [70%]). By day 100, 28 episodes of grade 3 liver toxicity were noted in 15 patients (table 2). No cases of grade 4 liver toxicity or veno-occlusive disease were identified. 120 separate grade 2–4 infectious events occurred in 44 patients (table 2); 49 grade 2 infections in 30 patients, 62 grade 3 infections in 40 patients, and nine grade 4 infections in nine patients. 59 episodes of grade 3–4 bacterial infection in 39 patients were recorded in the first 100 days, mainly during the neutropenic period. Three patients developed invasive fungal infections: aspergillosis in two and disseminated fusarium in one. Cytomegalovirus reactivation occurred in only five (16%) of 31 seropositive recipients. Other documented viral infections were rotavirus (five [10%]), adenovirus (one [2%]), human metapneumovirus (one [2%]), respiratory syncytial virus (one [2%]), influenza (one [2%]), BK virus (one [2%]), and herpes simplex virus (one [2%]).

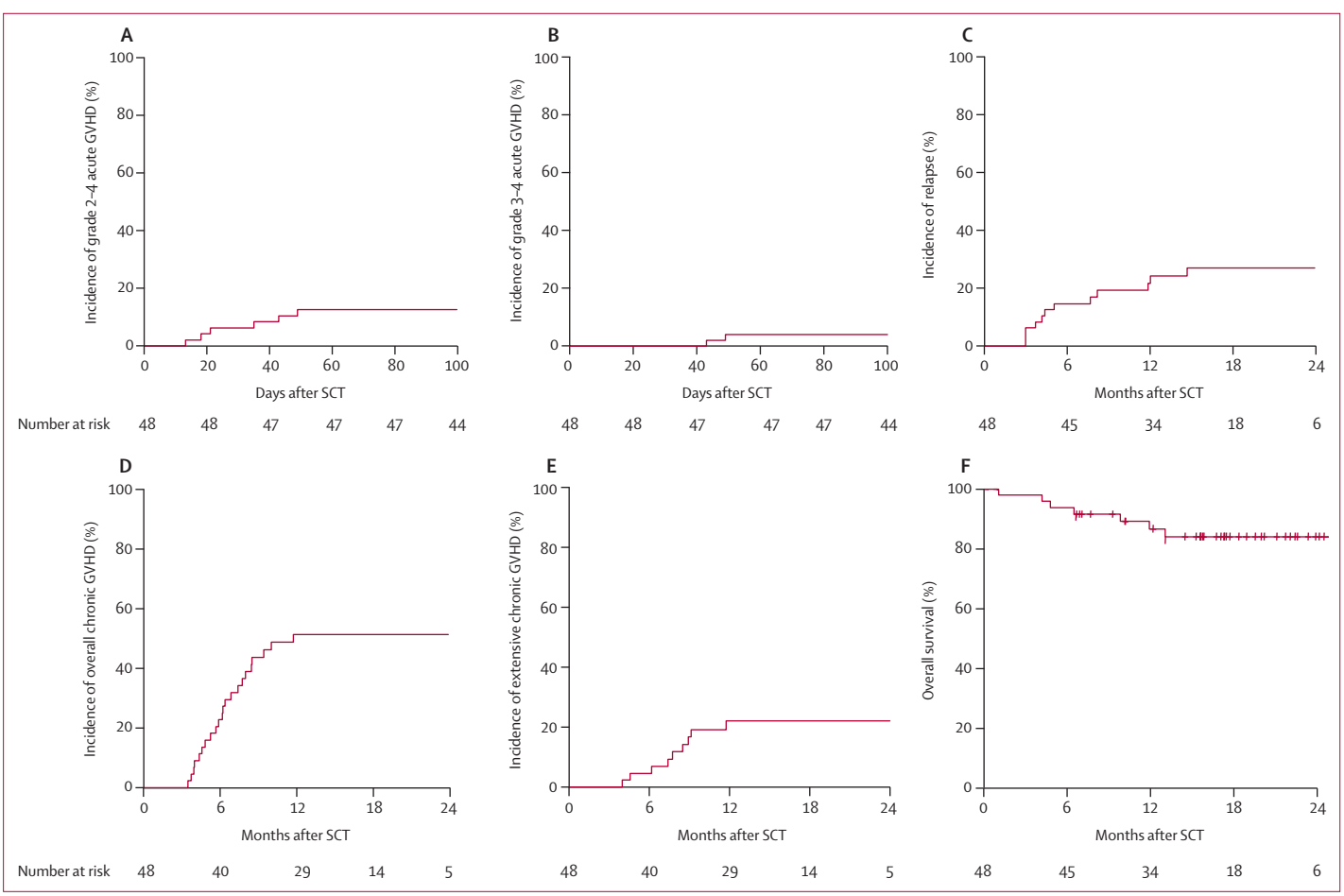

Figure 2: **Effect of interleukin-6 receptor inhibition on transplant outcome**
Effect of tocilizumab treatment on incidence of grade 2–4 acute GVHD (A), grade 3–4 acute GVHD (B), relapse (C), overall chronic GVHD (D), extensive chronic GVHD (E), and overall survival (F) in all 48 treated patients. GVHD=graft-versus-host disease. SCT=stem-cell transplantation.

Analysis of plasma cytokine concentrations shows that interleukin 6 concentrations were greatly increased (relative to baseline and the group of patients not treated with tocilizumab) between days 0 and 30 (figure 3A). High interleukin 6 levels are probably due to reduced consumption as a result of the low level of available interleukin-6 receptor in patients treated with tocilizumab. Interleukin 6 levels remained elevated at day 30 and returned to baseline by day 60, consistent with eventual antibody clearance. Soluble interleukin-6 receptor concentrations in untreated patients progressively decreased in the first 2 weeks after transplant; by contrast in patients who were treated with tocilizumab, soluble interleukin-6 receptor concentrations increased after allogeneic SCT, returning to baseline concentrations by day 60 (figure 3B). Soluble gp130 concentrations remained similar in treated and untreated patients and increased predominantly in response to transplantation (figure 3C). IFNγ and interleukin 8 concentrations seemed to be increased early after allogeneic SCT (figure 3D, E) in patients receiving interleukin-6 receptor monoclonal antibody, whereas concentrations of interleukin 17 (figure 3F) and TNF, interleukin 1β, and interleukin 4 (appendix p 7) in the plasma did not increase. Interestingly, concentrations of interleukin 17 increased in both groups beyond day 90 during the period in which chronic GVHD characteristically develops.

To understand the effect of interleukin 6 inhibition on immune reconstitution and function, we enumerated innate and adaptive cell populations in patients after allogeneic SCT. Interleukin-6 inhibition was associated with accelerated plasmacytoid dendritic cell and B-cell reconstitution (appendix p 8) with similar numbers of T-cell (appendix p 8) and myeloid dendritic cell subsets in the first 100 days (appendix p 8). Cytokine (IFNγ, interleukin 17A, TNF, and interleukin 4) protein expression was similar in CD4 T-cells in the presence or absence of interleukin-6 receptor inhibition 2 weeks after allogeneic SCT (appendix p 9). We performed microarray analysis to determine differential gene expression in monocytes, and confirmed differential regulation of a large number of molecules, including inhibition of SOCS3 (the downstream physiological inhibitor of STAT3 phosphorylation[18]), together with the interleukin-6 receptor and cytokines or chemokines and receptors involved in differentiation, inflammation, and migration (appendix p 10).[18–22]

The mRNA analysis of sort-purified donor CD4 T-cells quantified predetermined expression of genes associated with T-cell differentiation and function which are shown in appendix p 11. This analysis showed that SOCS3 mRNA was also reduced (and interleukin 18 increased) in patients treated with tocilizumab (appendix p 12). However, unsupervised hierarchical clustering did not delineate other interleukin-6 receptor monoclonal antibody-dependent changes, including T_{reg} (FoxP3), Th1 (t-bet), Th2, (GATA3), and Th17 (RORC) transcription factors or lineage-associated cytokines or chemokines (appendix p 12). Thus, the effects of interleukin-6 inhibition correlate with inhibition of the STAT3 pathway in monocytes and T-cells, but seem to be independent of T-cell differentiation in peripheral blood.

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Serious adverse events					
Infection (bacterial)	..	..	13*	6†	..
Infection (fungal)	..	..	..	2‡	..
Infection (viral)	..	..	2	..	..
Diffuse alveolar haemorrhage	..	..	..	1	..
Acute kidney injury	..	..	1	..	..
Multiorgan failure	..	..	..	1§	..
Pneumothorax	..	..	..	..	1§
Syncope	..	..	3	..	..
Diarrhoea	1	1	2	..	..
Delayed platelet engraftment	..	..	1¶	..	..
Adverse events‖					
Infection (bacterial)	..	18	39	1	..
Infection (fungal)	..	15**	1††	..	..
Infection (viral)	..	16	7‡‡	..	..
Hyperbilirubinaemia	..	..	5§§	..	..
Increased activity of liver enzymes	..	..	23¶¶	..	..
Diffuse alveolar haemorrhage	..	..	2	..	..
Pulmonary oedema	..	..	1§	..	..
Upper gastrointestinal haemorrhage	..	..	1§	..	..
Typhlitis	..	..	1	..	..
Oral mucositis	..	..	24	..	..
Nausea	..	..	1	..	..
Anorexia	..	..	1	..	..
Hypokalaemia	..	..	6	1	..
Hyperglycemia	..	..	7	..	..
Hypertriglyceridemia	..	..	1	..	..
Hypertension	..	..	2	..	..
Syncope	..	..	1	..	..

Data are number of events. Serious adverse events met one or more of the following criteria: required admission to hospital or prolongation of existing admission to hospital, resulted in a persistent or major disability or incapacity, was fatal or life-threatening, resulted in a congenital malformation, or required medical or surgical intervention to prevent the above outcomes. *Hospital admission with non-neutropenic fevers (three), line-related infection (three), sinusitis (two), bronchitis (one), urinary tract infection (one), leg ulcer (one), *Clostridium difficile* infection (one), and gastrointestinal infection (one). †Admission to intensive care unit with bacterial sepsis: *Klebsiella pneumoniae* (three), *Staphylococcus aureus* (two), and *Granulicatella adiacens* (one). ‡*Fusarium solani* sepsis (one) and invasive pulmonary aspergillosis (one). §These represent the same individual who developed *Staphyloccous aureus* sepsis, followed by acute kidney injury, pulmonary oedema, and finally bilateral pneumothoraces in the setting of respiratory syncytial virus infection. The patient had morbid obesity (body-mass index 62) and died from the consequences of an iatrogenic chest-wall haemorrhage. ¶Patient had a donor-specific HLA-DP antibody. ‖Toxicities are routinely encountered in all allogeneic stem-cell transplantation recipients and expected procedural grade 1–2 adverse events were not reported. All grade 3 or greater toxicities were reported. Additionally, all grade 2 or greater infections were also reported. **Oral candidiasis (12), vaginal candidiasis (two), and fusarium sinusitis (one). ††*Aspergillus terreus* skin infection. ‡‡Cytomegalovirus reactivation (four), rotavirus (two), and respiratory syncytial virus (one). §§All occurred within day 40, one patient also had grade 3 liver enzyme elevation. ¶¶Liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and γ glutamyltransferase) were considered separately. 23 events were noted in 11 patients, of which 15 events in eight patients occurred within day 40 (the period of exposure to tocilizumab).

Table 2: **Serious adverse events and adverse events up to day 100 after transplant**

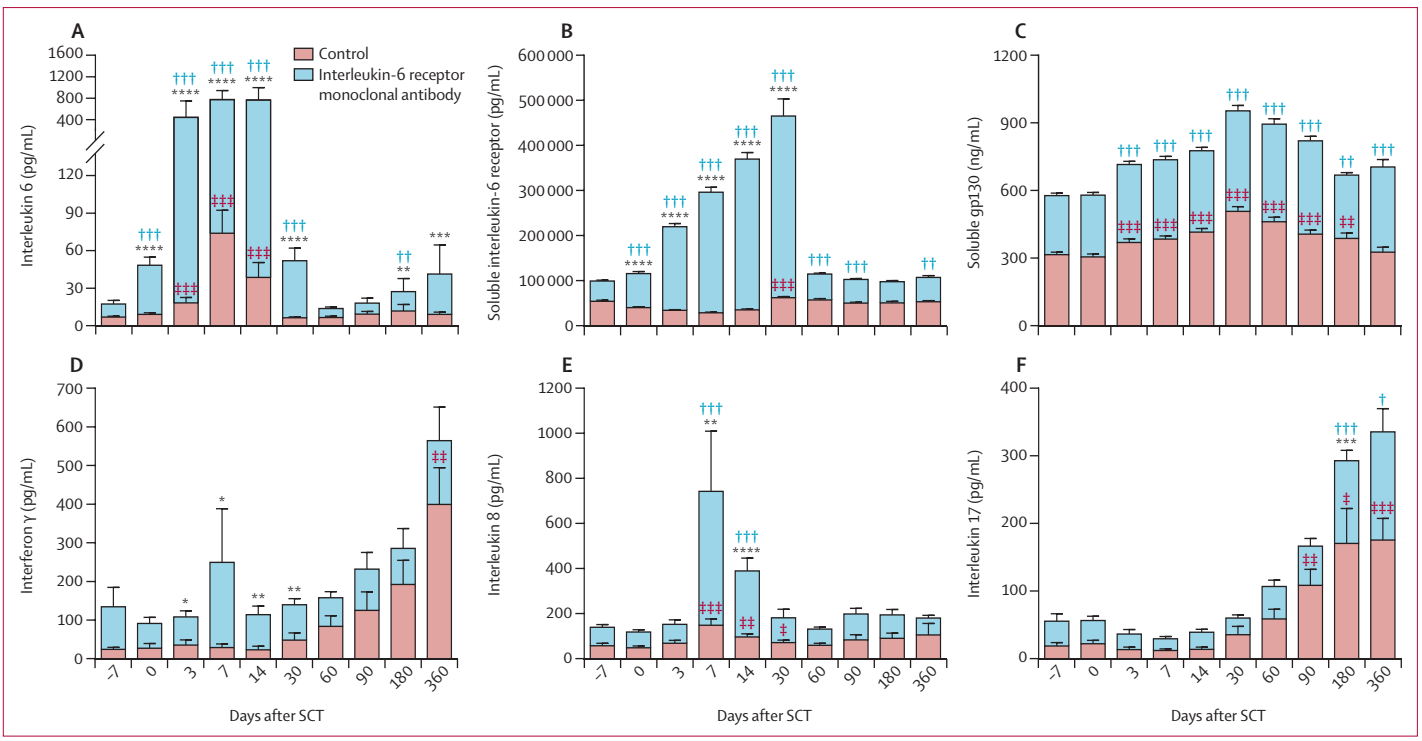

Figure 3: **Effect of interleukin-6 receptor inhibition on systemic cytokine concentrations with time**
Concentrations of interleukin 6 (A), soluble interleukin-6 receptor (B), soluble gp130 (C), interferon γ (D), interleukin 8 (E), and interleukin 17 (F) in plasma of 53 untreated patients compared with 48 patients treated with tocilizumab. ****p<0·0001, **p<0·01, *p<0·05, increased in interleukin-6 receptor monoclonal antibody-treated versus untreated (ie, blue *vs* red stacked bars). Data are mean (SEM, error bars). Blue ‡p<0·05, blue ‡‡p<0·01, blue ‡‡‡p<0·001, blue ‡‡‡‡p<0·0001, increased cytokine concentrations in interleukin-6 receptor monoclonal antibody-treated recipients versus respective baseline day –7 concentrations. Red †p<0·05, red ††p<0·01, red †††p<0·001, red ††††p<0·0001, increased cytokine concentrations in untreated recipients versus respective baseline day –7 concentrations.

Discussion

We show that addition of tocilizumab to standard GVHD prophylaxis for patients who have received HLA-matched allogeneic SCT is safe, and associated with a very low incidence of significant acute GVHD. This incidence was well below published data from our own and other centres for acute GVHD, which is between 40% and 60% in this setting (panel).[16,17]

The effector pathways inducing tissue injury in acute GVHD have been attributed to both cytokines and the cytolytic pathways of donor T-cell and NK cells. The relative contribution remains debated, but certainly inflammatory cytokines (interleukin 1, TNF, and IFNγ) play a major part in experimental systems in which GVHD is described as a cytokine storm.[1] Here, we show that interleukin 6 is the major detectable dysregulated and pathogenic cytokine early after clinical allogeneic SCT. With regards to the aforementioned cytokines, interleukin 1 neutralisation is not effective in preventing GVHD in randomised clinical trials.[2] By contrast, TNF inhibition seems to be effective,[23] but confirmatory randomised studies have not been done, probably because of the unknown ability to inhibit graft-versus-leukaemia effects.[24] Subsequent studies have also suggested efficacy for TNF inhibition in the initial[25] or third-line treatment of steroid refractory acute GVHD,[26] but there are inconsistencies such as restricted efficacy in some studies.[3] Here, we have shown that IFNγ does not seem to be systemically dysregulated in clinical allogeneic SCT recipients. Furthermore, the increase in IFNγ after interleukin-6 inhibition, despite a reduction in acute GVHD, could be consistent with the regulatory properties attributed to this cytokine[27] and is associated with high levels of protective immunity to cytomegalovirus.

The fact that clinical interleukin-6 dysregulation mirrors that observed in experimental systems suggests that most of the cytokine is generated in response to conditioning, and is not subject to inhibition by cyclosporin. Thus, inhibition of interleukin 6 and standard immune suppression seem to act synergistically, suggesting that interleukin-6 inhibition as an adjunct to GVHD prophylaxis is likely to be most effective after intensive conditioning. However, despite the decreased concentrations of interleukin 6 seen after reduced-intensity conditioning, interleukin-6 inhibition in this setting also led to a very low incidence of acute GVHD. Whether this is true after non-myeloablative conditioning remains to be determined. The incidence of chronic GVHD of any stage seemed similar to our own historical levels in this setting and published metadata.[17] This finding is intriguing in view of the effect on acute GVHD and the tight relationship between the development of

acute and chronic GVHD. Larger data sets with longer follow up are thus required to fully ascertain effects on chronic GVHD.[28] Likewise, although relapse was well within published norms,[17] further data are needed. Possibly, longer term interleukin-6 inhibition through tocilizumab treatment by repeated dosing could be needed to mediate an effect on chronic GVHD, but interleukin 6 does not seem to be systemically dysregulated beyond day 90 after allogeneic SCT when chronic GVHD manifests (concentrations beyond day 100 are not above baseline in controls). Importantly, acute GVHD, although T-cell dependent, is known to be mediated, at least in part, by inflammatory cytokines.[1] By contrast, the mechanistic pathway in chronic GVHD involves Th17 differentiation[29] and germinal centre B cells.[30] The increase in interleukin 17 noted late after transplant both in patients receiving interleukin-6 inhibition and controls is thus intriguing, and potentially explains why interleukin-6 inhibition does not seem to affect chronic GVHD. Thus, chronic GVHD and graft-versus-leukaemia could be interleukin 6-independent, the latter being consistent with data from in-vivo mouse studies.[5]

Finally, experimental studies suggest that interleukin 6 has direct pathogenic effects that mediate acute GVHD independently of CD4 T-cells in addition to its ability to promote pathogenic Th17 differentiation.[4,5] Despite the protective effects of interleukin-6 inhibition on acute GVHD and the inhibition of STAT3 signalling in monocytes and T-cells, we were unable to show quantitative or qualitative effects on T-cell differentiation in peripheral blood. By contrast, monocytes were highly modified, with transcriptional changes in multiple pathways. Although we cannot exclude effects of interleukin-6 inhibition on T-cell migration and differentiation specifically within GVHD target organs, our results argue that modification of myeloid cells and inhibition of interleukin 6-induced tissue damage directly could exert a major protective affect. Notably, the effects of cyclosporin almost certainly affect the ability of interleukin-6 receptor inhibition to alter T-cell differentiation and it is possible that alternative immune suppression (eg, with rapamycin) could modify this effect. Finally, and importantly, although all patients reported in our study were maintained on therapeutic cyclosporin to day 100, thereafter immune suppression was withdrawn at the discretion of the clinician. Thus, the cytokine and immune reconstitution data beyond day 100 are subject to influences by differential levels of immune suppression and should thus be interpreted with caution and this caveat in mind.

In summary, the addition of interleukin-6 inhibition to standard GVHD prophylaxis seems to be safe and results in a very low incidence of acute GVHD. Further testing in randomised, controlled trials is therefore warranted.

Panel: Research in context

Systematic review

We searched PubMed for articles published between June 1, 1950, and June 1, 2010, without language restrictions and using the keywords "graft versus host disease", "tocilizumab", "interleukin-6", and "hematopoietic stem-cell transplantation". In 2010, when planning the study and subsequently, no articles had been published that used interleukin-6 inhibition for prevention of graft-versus-host disease (GVHD) in patients undergoing haemopoietic stem-cell transplantation. However, at that time, we and others had published preclinical data suggesting that interleukin 6 was an important pathogeneic cytokine mediating GVHD.[4,5] Furthermore, we generated correlative observational clinical data showing interleukin-6 dysregulation early after transplant and together these findings justified and instructed the design of the phase 1/2 trial undertaken here. The study was thus designed to examine the ability of blocking interleukin-6 signalling early after transplant to attenuate the development of significant acute GVHD.

Interpretation

Our study represents to our knowledge, the first clinical trial examining the addition of tocilizumab to standard GVHD prophylaxis, and we show that it is safe and seems to reduce the incidence of moderate to severe acute GVHD. A randomised controlled trial of this therapeutic approach is now warranted.

Contributors

GAK designed the study, enrolled the patients, provided clinical care of patients in the study, analysed the data, and wrote the report. AV generated and analysed the patients and immunological data and wrote the report. SV, KHG, SDO, and ML generated and analysed the immunological data. PZ analysed the patients and immunological data. GT, LA, GB, LL, NC, and KPAM generated and analysed the mRNA data. JL, ES, and JA coordinated and collected the patient samples and data. AKM, AJM, STSD, ES, JPB, and CIC enrolled and provided clinical care of patients in the study. CH did the engraftment analysis. SKT enrolled and provided clinical care of patients in the study, analysed the data, and wrote the report. GRH designed the study, and enrolled and provided clinical care of patients in the study, analysed the data, and wrote the report.

Declaration of interests

We declare no competing interests.

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