Progress in treating chronic granulomatous disease

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Summary

Chronic granulomatous disease is a primary immunodeficiency due to a defect in one of six subunits that make up the nicotinamide adenine dinucleotide phosphate oxidase complex. The most commonly defective protein, gp91phox, is inherited in an X-linked fashion; other defects have autosomal recessive inheritance. Bacterial and fungal infections are common presentations, although inflammatory complications are increasingly recognized as a significant cause of morbidity and are challenging to treat. Haematopoietic stem cell transplantation offers cure from the disease with improved quality of life; overall survival in the current era is around 85%, with most achieving long-term cure free of medication. More recently, gene therapy is emerging as an alternative approach. Results using gammaretroviral vectors were disappointing with genotoxicity and loss of efficacy, but preliminary results using lentiviral vectors are extremely encouraging.

Keywords: chronic granulomatous disease, inflammation, infection, haematopoietic stem cell transplantation, gene therapy.

Chronic granulomatous disease (CGD) was first described in 1954 in children with elevated serum gamma globulin levels who suffered from recurrent infections.

The most common defects in out-bred populations are in CYBB, which is X-linked. Other defects are inherited in an autosomal recessive fashion and are more common in areas of high consanguinity. CYBA encodes p22phox, which is the other membrane-bound cytochrome component embedded with gp91phox within the membrane of lysosomes. These subunits come together with four cytosolic components, p47phox, p40phox, p40phox, p40phox. Rac2 and Rap1 are also required for activation and assembly of the functional complex. The assembled cytochrome is stabilised in the endoplasmic reticulum membrane by a protein, 'essential for reactive oxygen species' (EROS), encoded by CYBC1. Defects in CYBC1 also cause CGD. Within the phagocyte lysosome, activation of NADPH oxidase leads to loss of an electron, which is transferred to molecular oxygen, creating a negative charge and forming superoxide. Superoxide spontaneously, or through superoxide dismutase, converts to hydrogen peroxide, which reacts with superoxide anion to form a highly reactive hydroxyl radical that converts to hypochlorous acid in the presence of myeloperoxidase and chlorine. Phagocyte production of these reactive oxygen species enables potassium and proton influx into the phagolysosome, which activates granule proteases such as cathepsin G and elastase. The proteases cause destruction of phagocytosed microorganisms. Superoxide has a dual role of activating lysosomal molecules to induce killing and a direct microbicidal affect.

Molecular genetics

Chronic granulomatous disease occurs because of defects in the oxidative burst accompanying phagocytosis in myeloid cells. Defects arise in one of five subunits which make up nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, important for the catalytic conversion of oxygen to generate oxygen free-radical superoxide (O2·−), which gives rise to H2O2, HOCl and hydroxyl radicals that are critical for killing pathogenic bacteria and fungi. In the resting state, NADPH exists as two components:

- a membrane-bound heterodimer embedded within lysosomal membranes, that is composed of gp91phox and p22phox encoded by CYBB and CYBA, respectively;
- cytosolic proteins p47phox, p67phox, and p40phox encoded by NCF1, NCF2 and NCF4, respectively.

CGD is estimated to affect approximately 1 in 130,000 to 250,000 live births in an out-bred population, although...
this number may be considerably higher in populations with a high degree of consanguinity. Diagnosis tends to be at a younger age in X-linked disease and in male patients. The clinical presentations of patients with CGD are predominantly infectious or inflammatory (Table I). Common pathogens include Staphylococcus aureus, Burkholderia cepacia complex (which is characteristic of CGD), and Burkholderia cepacia complex bacteriaemia is pathognomonic), Serratia marcescens and Nocardia species, as well as mycobacterial species including atypical species and BCG. Aspergillus species, particularly Aspergillus fumigatus are well recognised and are the most common cause of death, but other fungal pathogens are also described (Table I). Worldwide, relatively few organisms are responsible for the majority of infections. Higher than expected rates of M. Tuberculosis may be seen in CGD patients living in TB endemic areas.

In children, infection is the most common presentation. Suppurative adenitis, pneumonia, liver and lung abscesses are the most common infectious presentations. Most liver abscesses are caused by Staphylococcus aureus. Often, there is a significant inflammatory element associated with infection. Successful resolution can be obtained using antimicrobial therapy in conjunction with systemic cortico-steroids. A particularly aggressive form of pneumonitis associated with CGD, mulch pneumonitis, is a fulminant pneumonitis after exposure to aerosolised fungal pathogens. Combination treatment with anti-fungal agents and cortico-steroids has led to successful resolution (Fig 1).

Although infection was initially recognised as an important presenting feature, it has been increasingly recognised that inflammatory manifestations play a significant role in the complications of CGD. Most common is inflammatory bowel disease, which may mimic Crohn’s disease, although granulomatous strictures may cause oesophageal, pyloric or small bowel obstruction. Inflammatory lung disease is also a feature of CGD. The presence of granuloma can be a complicating feature, as mycobacterial infection (tuberculosis or atypical mycobacteria) are well described.

### Table I. Bacterial and fungal pathogens seen in chronic granulomatous
disease.

<table>
<thead>
<tr>
<th>Bacteria</th>
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<tr>
<td>Staphylococcus aureus</td>
<td>Aspergillus fumigatus</td>
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<tr>
<td>Burkholderia cepacia complex</td>
<td>Aspergillus nidulans</td>
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<tr>
<td>Haemophilus spp</td>
<td>Other Aspergillus spp</td>
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<tr>
<td>Mycobacterium tuberculosis</td>
<td>Candida spp</td>
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<tr>
<td>Atypical Mycobacteria spp (including BCG)</td>
<td>Mucor spp</td>
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<tr>
<td>Nocardia spp</td>
<td>Acremonium spp</td>
</tr>
<tr>
<td>Serratia marcescens</td>
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<tr>
<td>Klebsiella pneumoniae</td>
<td></td>
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<tr>
<td>Salmonella spp</td>
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<td>Actinomyces spp</td>
<td></td>
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<tr>
<td>Acinetobacter spp</td>
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Inflammatory complications in CGD are due to an aberrant response to inflammatory stimuli. Patients with CGD are susceptible to granuloma formation, which affect any hollow viscus, but particularly the gastrointestinal and genitourinary tracts, as well as lungs. The exact inflammatory pathology is poorly understood, but several pathways are likely to contribute. Failure of clearance of phagocytosed material is likely to be an important inflammatory stimulus. CGD phagocytes that lack functional NADPH oxidase accumulate but are unable to clear microbial material or cellular debris, including apoptosed neutrophils, resulting in persistent cell activation and exaggerated inflammation. Inflammase activation leading to caspase-1-mediated inflammation may be important in CGD-associated inflammatory response. Symptomatic CGD patients have significantly-raised monocyte-derived IL1β—particularly in those with colitis, but seen in all symptomatic CGD patients compared to healthy controls. Defective autophagy may be seen in both mice and human CGD patients, and is associated with reactive oxygen species-independent activation of the inflammasome and increased release of IL1β.

Indoleamine 2,3-dioxygenase may have an important role in the exaggerated inflammation typical of CGD. p47phox knock-out mouse mice infected with Aspergillus fumigatus had more damaging inflammatory lung injury than the initial infection as well as inefficient tryptophan catabolism as a result of blocked indoleamine 2,3-dioxygenase function.

Most cases of CGD are recognised in childhood, although adults with CGD are increasingly being treated. Some of these were children treated for CGD and now transitioned to adult care, whereas others were only diagnosed in adulthood or only presented in adulthood for the first time. Infectious and inflammatory complications are similar to those seen in childhood, but are often more severe, and require
intervention and hospitalisation; pulmonary and gastrointestinal complications are most common.9,19,20

**Diagnosis**

Diagnosis is confirmed by the demonstration of abnormal NADPH oxidase activity or protein expression, an inability of phagocytes to produce reactive oxygen species and subsequent identification of the specific genetic mutation. Diagnosis may be made before the onset of symptoms when there is a positive family history. NADPH oxidase activity may be assessed in a number of ways: through superoxide generation, hydrogen peroxide production or oxygen consumption.

As NADPH oxidase is inactive in resting phagocytes, patient neutrophils are activated by a stimulus, such as phorbol-12-Myristate-13 Acetate, to produce reactive oxygen species, such as hydrogen peroxide. Superoxide generation may be measured by examining the ability of phagocytes to reduce a known reagent—for example, nitroblue tetrazolium (NBT) in the NBT reduction test. Nitroblue tetrazolium is a yellow dye that is reduced to blue formazan by the superoxide produced by the respiratory burst. The intracellular reaction result is read manually. CGD phagocytes are unable to generate superoxide and remain yellow as they do not reduce NBT. Normal phagocytes show the blue insoluble, precipitate of formazan. X-linked CGD carriers demonstrate both colours, as two populations of phagocytes are present. Interpretation of the result is subjective and detection of X-linked CGD carriers can be difficult as the two phagocyte populations may not be well defined or distinct. However, an experienced technician will be able to quantify the percentage of functioning neutrophils. Flow cytometry may be used to demonstrate absence or reduction in the oxidative burst in CGD patients and carriers. Neutrophils are isolated and stimulated in the same manner as for the NBT reduction test. An H₂O₂ detecting agent such as dihydrorhodamine (DHR)-1,2,3 is added and freely enters the cells. DHR is oxidised by the reactive oxygen species to rhodamine-1,2,3, which emits a fluorescent signal detected by the flow cytometer.

Measurement of oxygen consumption is rarely used in clinical practice as it is time-consuming and expensive, but does provide a quantitative assessment of oxygen consumption by use of an oxygen electrode.

Determining which NADPH oxidase component is defective when an abnormal oxidase burst has been recorded can be achieved by looking at protein expression of individual sub units. X-linked disease due to defective gp91phox may be inferred with a positive family history of X-linked inheritance and demonstration of a dual population of phagocytes in the maternal carrier, as long as the genetic defect does not solely affect germline cells. Flow cytomteric analysis of intact neutrophils using a monoclonal antibody against NADPH oxidase components may determine the presence or absence of the protein. However, the membrane components of NADPH oxidase, gp91phox and p22phox, stabilise each other, so if one is absent the other is not detectable.

Genetic analysis should be performed in all cases of CGD,21 as defining the mutation allows for certainty in diagnosis and more accurate genetic counselling. The analysis of NCF1, encoding p47phox, is complicated by the presence of pseudo-NCF1 genes either side of the NCF1 locus.

Precise genetic diagnosis may have implications within the clinical setting. For patients undergoing HSCT from a female donor, the donor may be accurately screened to ensure they do not carry the defective gene, particularly if they are a female relative and carry CYBB mutations. If gene therapy is considered, it is imperative that there is an accurate genetic diagnosis. The precise mutation may be used to determine association with degree of reduction in NADPH oxidase activity, information that can be transferred into the clinical setting.

**Conventional treatment**

*Prophylaxis*

The main focus of conventional treatment for CGD is prevention and management of infections and inflammatory complications. Widespread antibiotic prophylaxis was introduced from the 1970s;22 sulphamethoxazole-trimethoprim is preferred because of its safety and efficacy, and because of the intracellular action. It was effective at reducing surgical interventions and length of hospitalisation,23 although overall survival was not altered due to deaths from fungal infection.24 The introduction of itraconazole prophylaxis, with particular efficacy against aspergillus species, has improved survival as well as reducing hospitalisations.25–28 However, resistant organisms, sub-therapeutic levels due to poor absorption secondary to inflammatory bowel disease and poor compliance mean that fungal infections remain a significant cause of morbidity and mortality.

The use of interferon gamma as routine prophylaxis has been controversial. It has been adopted more widely in the United States than it has in Europe. Early studies suggested that it was able to enhance superoxide production at a cellular level,29,30 although other studies failed to replicate these findings.31 A multicentre study demonstrated a 70% reduction in incidence of infections in the treated group compared to placebo, who received antibiotic prophylaxis alone,32 although the rate of infections in an antibiotic-only treated cohort in Europe was lower than in the interferon gamma-treated group.33 Most benefit may be in those with splice site mutations,34 and there appears to be no additional benefit in those who receive regular antibiotic and antifungal prophylaxis.26

*Treatment of infection*

Aggressive and prompt use of appropriate antibiotics and antifungals remains the most important basis of treatment of
infections. These should be empirically directed against organisms associated with CGD until cultures are confirmed. A high index of suspicion for fungal infection should be entertained if symptoms, particularly fever, persist despite treatment. A tissue sample may be required to identify the organism (Fig 1). Serum markers such as galactomannan are of limited value in CGD, but may be more helpful if found on a targeted broncho-alveolar lavage. Serial, rather than single, samples are more likely to be useful. Voriconazole or posaconazole are recommended for the treatment of invasive fungal infection due to Aspergillus species and other fungi associated with CGD, but treatment should be guided by culture and sensitivity results. Serum levels should be measured to ensure that therapeutic levels are obtained; bowel inflammation may make drug absorption more erratic. Serious side effects have been reported. Photosensitivity may be an issue with voriconazole, and skin malignancies have been reported following prolonged use. Neurological toxicity and periostitis have rarely been reported with long-term use, the latter occasionally in association with hyperflourosis. Hepatic toxicity is common with all triazoles. More rarely, primary adrenal insufficiency due to direct inhibition of the steroidogenesis pathway has been reported. Dual therapy is not recommended, although, in reality, many physicians may consider it when a patient is extremely sick. Surgery may be required for complete resolution of symptoms, but treatment should be entertained if symptoms, particularly fever, persist despite treatment. Surgical intervention can be an important adjuvant treatment, particularly to debride invasive fungal disease, and tissue samples may aid in identification of the organism and antifungal sensitivities. The outcome of thoracic surgery in 35 CGD patients with severe pulmonary infection has been reported, with a 90-day mortality of 6% and 37% mortality at time of analysis, predominantly from pulmonary infection. Poor prognostic features were noted, namely chest wall resection or significant intra-operative blood loss. The authors concluded that patients requiring thoracic surgery were high-risk and should be considered for definitive treatment following surgery.

Anti-inflammatory treatment

Inflammatory complications, particularly colitis, are particularly challenging symptoms to address in patients with CGD, as treatment with immunosuppressive agents might increase the risk of infection. Cortico-steroids do not appear to increase the bacterial infection risk, although there is an increased risk of fungal infection, as well as the recognized complications of long-term use and, particularly, growth failure. Steroid-sparing agents have been used, particularly in CGD colitis, including azathioprine and sulfasalazine. Thalidomide has also been used with some success to treat colitis. Pioglitazone is a peroxisome proliferator-activated receptor gamma (PPARγ) agonist that is used to treat type 2 diabetes. One study demonstrated, in murine models and human cells, that pioglitazone enhanced stimulated mitochondrial-derived reactive oxygen species production in circulating neutrophils and blood monocytes and in neutrophils and inflammatory macrophages recruited to inflammatory tissues. It was concluded that pioglitazone may be considered as a possible new treatment strategy for CGD patients with severe infection. A subsequent trial in a small number of patients failed to demonstrate any clinical benefit.

Identification of the role of pro-inflammatory cytokines in the inflammatory process in CGD has facilitated the adoption of targeted blockade as a focused treatment. Anti-TNF monoclonal antibodies have been used, usually in association with other immunosuppressive agents, and most usually for treatment-resistant or fistulating colitis. Infliximab is most commonly used, but there are increased risks of infection. Antimicrobial prophylaxis and careful monitoring for possible infections are required and it is not recommended for maintenance of remission in CGD. Use of adalimumab has been reported in CGD patients with severe refractory colitis. Treatment with the recombinant IL-1 receptor-targeted antagonist anakinra demonstrated therapeutic benefit in some patients with severe colitis. However, other patients with severe refractory colitis experienced only marginal or no benefit, and no long-term relief. Although treatment was administered for at least 3 months, no significant infectious complications were encountered, however. There is a single case report documenting use of the IL-23 antagonist ustekinumab to treat longstanding treatment-recalcitrant CGD colitis. The patient demonstrated significant symptomatic improvement, but developed a probably severe infection requiring discontinuation of ustekinumab, after which symptoms returned. Vedolizumab is a monoclonal antibody that binds to integrin α4β7 heterodimer and blocks...
interaction of α4β7 integrin with MAdCAM-1, which prevents leukocyte binding to endothelial surface and its extravasation into inflamed tissue. It is used to treat ulcerative colitis and Crohn’s disease. A case report demonstrated efficacy in a patient with fistulating colitis. However, one small study of use in 11 patients with CGD is published with more detailed follow-up. Seven patients reported subjective clinical improvement; but although more than half of patients had mucosal improvement demonstrated with interim endoscopy treatment, they failed to sustain mucosal healing, and there was no steroid-sparing effect.

One study focused on ways to counter the defect in autophagy and the associated inflammasome activation manifest in NOX defective mononuclear phagocytes. Patients with or without inflammatory manifestations had a reproducible inflammatory phenotype typified by

- an increase in non-classic and intermediate monocytes
- a pro-inflammatory state of mononuclear phagocytes with increased IL-1β and tumour necrosis factor alpha (TNFα) content
- a bias of CD4+ T lymphocytes toward a TH17 phenotype
- an increase in IL-17A–secreting neutrophils.

Studies of mononuclear phagocytes collected from patients with clinical inflammation, and treated in vitro with the mechanistic target of rapamycin (mTOR) inhibitor rapamycin to restore autophagy, showed reduced basal TNFα production and secretion, suppression of IL-1β, IL-6 and IL-23 secretion by lipopolysaccharide-primed monocytes and impaired TH17 switching. Importantly, inhibition of the IL-1 receptor with anakinra enhanced the inhibition of phagocyte IL-1β secretion by rapamycin. Whilst these are in vitro data, they suggest that a combination of rapamycin with anakinra could be considered for patients with CGD with inflammatory complications.

As increasing numbers of biological treatments become available, studies are required to delineate which agents, and in which combinations, lead to infection-free clinical benefit.

**Stem cell therapies**

*Haematopoietic STEM CELL transplantation*

The introduction of prophylaxis leads to improved outcomes. A cohort of 21 children, followed for a decade at a single centre of excellence, documented no fungal infections or deaths and acceptable growth. Nonetheless, although conventional and new biological treatments are improving the mortality risk for patients with CGD, fungal infection remains an important cause of mortality and morbidity, and the fundamental problem of genetically-defective haematopoietic stem cells persists. Patients remain at risk of developing life-threatening infection or inflammation as they age. In the UK cohort study, patients were frequently hospitalised and required surgery every 4–5 years. Furthermore, complications were more common after the diagnosis of CGD was made. Cumulative survival was 88% by 10 years, 73% by 20 years and 55% by 35 years of age.

Haematopoietic stem cell transplantation (HSCT) for patients with CGD began in the early 1970s, but was sporadic and considered experimental. Although CGD is a life-threatening and potentially life-limiting primary immunodeficiency, the decision to take a patient with CGD to transplant is not straightforward. Early experiences of rejection and graft failure demonstrated the need for significant pre-transplant cytoreductive chemo-conditioning and immunosuppression to reduce the risk of rejection. Patients with CGD have a normal lymphocyte response and many have hyperinflammation, increasing the likelihood of rejection. However, early full-dose busulphan/cyclophosphamide-based conditioning regimens have significant short-term toxicities (including infection, increased risk of graft versus host disease (GvHD), veno-occlusive disease and mortality) and long-term toxicities (including infertility). The decision on whether to take a patient that was well on prophylaxis to a potentially-curative transplant, but with associated toxicities and potential mortality, was difficult for transplant physicians and families. A study at the National Institutes of Health, Bethesda, attempted to minimize these toxicities. Ten patients (eight with X-linked disease) aged 5–36 years (median 15 years) received a conditioning regimen comprised of cyclophosphamide 60 mg/kg (on day −7 and −6), fludarabine 25 mg/m² (day −5 to −1) and antithymocyte globulin (ATG) 10 mg/kg (day −5 to −2) and ciclosporin as GvHD prophylaxis. The patients had previously experienced two or more life-threatening infections requiring intravenous antibiotic treatment but were infection-free at the time of stem-cell transplantation. Donors were all HLA-identical siblings and the stem cell source was G-CSF mobilized peripheral blood stem cells that were CD34+ selected using magnetic beads to reduce the risk of graft versus host disease. The reduced intensity conditioning regimen and removal of a potential alloreactive graft versus marrow effect by donor T-lymphocytes reduced the ease of engraftment. Nevertheless, 90% of patients engrafted, albeit with use of between one and five donor T-lymphocyte infusions. Four patients developed grade I–IV GvHD, and two developed chronic GvHD. At time of publication, seven were alive: one with on-going chronic GvHD and six were well and disease-free. The three deaths were related to infection, GvHD and complications of a second stem cell procedure in one patient.

A multi-centre study by the European Inborn Errors Working Party (IEWP) of ESID and EBMT reported on transplants using myelo-ablative conditioning and an unmodified stem cell source. Fourteen centres contributed 27 patients, 25 of whom were children, 23 male, 22 with X-linked disease and one a female carrier with extreme lyonisation. Patients were grouped into those with no overt infection or inflammation (11), those with active inflammation/
inflammatory sequelae (seven) and those with treatment-refractory infection (nine) at time of transplant. Twenty-five patients received HLA-identical matched sibling transplants, five from heterozygous CGD carriers. Two patients (with no overt infection or inflammation) received an HLA-identical unrelated donor transplant. Twenty-three patients received a busulphan-based myeloablative (16–20 mg/kg) conditioning regimen, mostly combined with cyclophosphamide 200 mg/kg. Lower-intensity conditioning regimens were used in four sick patients, two with active therapy-refractory infection and two with poor lung function.

Twenty-four patients received full, T-lymphocyte-replete marrow innocula, whilst three patients receiving a lower-intensity conditioning regimen were given G-CSF-mobilised, T-lymphocyte-replete peripheral blood stem cells; all patients received ciclosporin prophylaxis. Twenty-two evaluable patients of 23 who had received HLA-identical matched sibling transplants achieved full donor chimerism; one died before engraftment. Of four patients who received lower-intensity conditioning (two bone marrow, two PBSC), two achieved full donor chimerism and two failed to engraft. In those with no overt infection or inflammation, one developed grade II GvHD which resolved; all had full donor chimerism. Of those with active inflammation/inflammatory sequelae at time of transplant, three developed GvHD: grade II skin that resolved (two), and grade IV gut GvHD in one that progressed to chronic GvHD; all patients survived, with full (six) or high level (one) donor chimerism. Of the nine patients with treatment-refractory infection at time of transplant, three developed grade II-IV acute GvHD, of whom one died. The two survivors of acute GvHD developed chronic GvHD. Two of the nine patients failed to engraft and died, and one other died before engraftment. Six patients developed full donor chimerism, of whom one died. Infections and inflammatory lesions in the five survivors resolved. The deaths occurred in patients with pre-existing treatment-refractory fungal infections—also the group in which the most severe GvHD occurred. Use of myeloablative conditioning and lymphocyte replete stem cell inocula facilitated complete donor chimerism and was generally well-tolerated. In some patients, pulmonary restriction improved. The authors concluded that excellent survival was achievable using HLA-genoidentical donors for HSCT in patients with active inflammation or organ dysfunction due to chronic inflammation. Additionally, CGD patients with an HLA-identical sibling and a history of recurrent invasive infections or inflammatory, steroid-dependent disease, should be considered for HSCT, before irreversible organ damage arises.

A subsequent single-centre UK study examined transplant results in 20 consecutive patients transplanted between 1998 and 2007.69 Nineteen had X-linked disease; age at transplant ranged between 15 months and 21 years, median 75 months. Ten received stem cells from an HLA-identical sibling (one sibling umbilical cord blood stem cells).70 Eight patients received 10/10 HLA-identical unrelated donor stem cells (two PBSC, six BM); one received 9/10 HLA-matched (C mismatch) unrelated donor PBSC; one received 9/10 HLA-matched (A mismatch) unrelated donor umbilical cord blood stem cells. Sixteen patients received myeloablative conditioning (busulphan 16 mg/kg, cyclophosphamide 200 mg/kg) with alemtuzumab (1 mg/kg), given if an unrelated donor was used. Two patients with active fungal infection and restrictive pulmonary disease respectively received fludarabine 150 mg/m², melphalan 140 mg/m² and alemtuzumab 1 mg/kg. One adult patient received alemtuzumab 2 mg/kg, fludarabine 150 mg/m² and busulphan 8 mg/kg, whilst the other adult patient received CAMPATH 1-G (100 mg total dose), busulphan 16 mg/kg and melphalan 140 mg/m². All patients received cyclosporine as GvHD prophylaxis. Ten patients had active inflammatory disease and two had active fungal infection at time of transplant. Eighteen patients survived, with a follow-up of 4–117 months (median 61). Engraftment with functioning neutrophils was observed in 19 patients. The oxidative burst became normal or ≥70% of neutrophils in 15 patients. Two required unconditioned boost marrow infusions from the original donor after the first HSCT to improve falling donor chimerism. One patient had a stable oxidative burst of 45%. Patients were able to discontinue antimicrobial prophylaxis and there were no significant infections beyond 3 months post-transplant. Patients demonstrated resolution of inflammatory lung disease and colitis and achieved catch-up growth post-transplant. One patient from the HLA-identical sibling and one from the unrelated donor group died; both had active fungal disease. This study demonstrated good outcomes when using well-matched unrelated donors, as well as matched sibling donors, and in particular showed resolution of inflammation and restoration of growth. Further study of the UK paediatric CGD cohort in the modern transplant era demonstrated the same good survival (90%) in transplanted patients and those receiving antibacterial and antifungal prophylaxis.71 Notably, however, those patients who received transplant for their disease had a significantly better quality of life than non-transplanted patients, similar to that of age-matched normal control,72 although this finding was not replicated in a smaller Italian study of 47 patients.73

Concerns about sequelae of HSCT and, in particular, adverse events related to myeloablative chemotherapy remained an obstacle in offering HSCT to affected families. However, conventional reduced intensity conditioning generally lead to poor engraftment or high levels of GvHD.74,75

In a landmark multicentre study, the efficacy and safety of a targeted sub-myeloablative busulphan-based regimen was assessed.76,77 In this prospective study, 56 patients (34 with X-linked disease) from 16 centres, median age 12–6 years, were studied. Thirteen patients were ≥18 years (range 18–39; median 22). Seventy-five percent of patients were high-risk, with microbiologically-proven active infections, mainly caused by Aspergillus spp. and autoinflammatory complications, predominantly colitis. The reduced-intensity
conditioning regimen comprised fludarabine immunosuppressive chemotherapy, rabbit ATG-Fresenius or alemtuzumab serotherapy and low-dose or targeted busulfan, targeting busulfan exposure to a cumulative area-under-the-curve (AUC) value of 45–65 mg/l × h, equivalent to 55–75% of the full myeloablative cumulative AUC of 80–100 mg/l × h. Twenty-one patients received stem cells from HLA-identical related donors; 35 were transplanted using an HLA-matched unrelated donor, of which 25 were HLA-10/10 matched and 10 were HLA-9/10 matched. There were no significant acute conditioning-related toxicities. The overall survival was 93% and event-free survival was 89% (50 of 56).

Three patients had early or late graft failure. The cumulative incidence of grade III–IV GvHD was 4%; chronic GvHD was 7%. Stable myeloid donor chimerism (≥90%) was recorded in 52 (93%) surviving patients. These results, using a safer reduced intensity conditioning regimen, demonstrated excellent survival in a mixed population of children and adults, many of whom had significant CGD-related complications at time of transplant, with durable high level donor chimerism.

Treosulfan is a water-soluble bi-functional alkylator that has myeloablative and pronounced immunosuppressive properties, enabling stable engraftment post-transplant. It has reduced organ toxicity compared to high-dose busulfan and cyclophosphamide, causing less acute toxicity post-transplant. The outcome of treosulfan-based conditioning of 70 CGD patients from 16 centres worldwide was reported on behalf of the ESID/EBMT-IEWPH. Median age at transplant was 107 months (range 46–232); 66 patients were male. Fifty-six had X-linked CGD. Sixty-four patients had high-risk criteria, as ongoing or previous radiologically and microbiologically-proven infection or autoinflammation. Thirteen patients received stem cells from a 10/10 HLA-matched related donor, whilst 56 received the transplant from a matched unrelated donor (44 10/10 matched, 12 9/10, one 4/6 umbilical cord blood cells; one received a CD3+ TCRαβ + T-lymphocyte depleted haplo-identical parental transplant). Treosulfan was the primary myeloablative agent; 66% of patients received treosulfan, fludarabine and serotherapy with either ATG or alemtuzumab, whilst 24 received other regimens, with 15 patients receiving treosulfan, fludarabine, thiopeta, with ATG or alemtuzumab. The overall survival was 91.4%; 64 patients were alive with a median follow-up of 34 months (13–102). The cumulative incidence of grade III–IV GvHD was 12%.

Eight patients required second procedures, including two boost infusions from the original donor without further conditioning, three donor lymphocyte infusions and five second transplants, giving an event free survival of 81.4%. Out of 64 patients where data were available, donor myeloid chimerism was higher than 95% in 51 (80%). These results demonstrated that use of a reduced toxicity myeloablative regimen led to good survival and donor chimerism, although some second procedures were needed.

A recently published multicentre retrospective study by the EBMT/ESID IEWP documents the largest CGD transplant cohort to date. The study describes 712 patients (75% X-linked, 635 children <18 years) with CGD undergoing allogeneic HSCT between 1993 and 2018; 87% of transplants were after 2006. Many patients had significant disease before transplant, including infections (68%), colitis (24%) and liver impairment (9%). Busulphan-based conditioning regimens were employed in 60% of patients, treosulfan-based in 20%. Eighty percent of patients received a graft from a matched related or unrelated donor, 20% from a mismatched donor. Median age at transplant was 7 years (range 0–1–48.6). Median follow-up was 45 months (range 17–64–82.79). Overall survival was 85.7%. Older age at transplant (P = 0.0001) and use of 9/10 HLA-matched donors (0.01) associated with reduced survival. A trend for reduced survival was seen in patients with pre-existing colitis (P = 0.052), a finding at variance with experience in the U.S., where 49/145 transplanted patients with CGD had inflammatory bowel disease and survival between the groups was the same. In the IEWP study, the conditioning regimen did not impact overall survival. The authors conclude that there was an excellent outcome after HSCT for patients with CGD, and that transplant should be firmly considered for younger patients, especially if there was a well-matched donor. However, a single-centre UK study has also demonstrated excellent outcomes using CD3+ TCRαβ + T-lymphocyte depleted haplo-identical donors (although results using post-transplant cyclophosphamide have not been so encouraging). Recent studies documenting medical problems in carriers of X-linked CGD have led to recommendations not to use them as donors for CGD patients if possible. Although transplant outcomes are best in younger patients, nevertheless, good outcomes can be demonstrated in adolescent and adult patients. An 82% survival was documented in 11 patients, age 17–28 years (median 19), all of whom had significant pre-existing infectious or inflammatory complications, using matched, family or unrelated or one antigen mismatched unrelated donors. Seven adolescents, six with high-risk disease, were successfully transplanted using reduced-toxicity myeloablative busulfan conditioning—although one had graft failure and was re-transplanted, and another had grade IV skin and gut GvHD. All had full donor myeloid chimerism and disease resolution.

A particular group of patients in whom HSCT can be challenging is those with McLeod phenotype (a neuroacanthocytosis syndrome) and X-linked CGD caused by large deletions on the X-chromosome that encompass CYBB and XK. XK encodes the Kx transmembrane protein, which links to the Kell glycoprotein on erythrocytes. The McLeod blood phenotype is rare and characterized by reduced Kell glycoprotein antigen expression. Patients develop an allo-immune response following erythrocyte or granulocyte transfections and may develop brisk haemolysis as a consequence of incompatible transfusions through HSCT. A French study describes eight patients, four of whom were transplanted, three of whom survived.
<table>
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<th>CR</th>
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<td>HSCT</td>
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<tr>
<td>Goudemand62</td>
<td>1</td>
<td>Cy</td>
<td>BM</td>
<td>no</td>
<td>Yes</td>
<td>1.5</td>
<td>Rejection at 2 months, A + W</td>
</tr>
<tr>
<td></td>
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<td>ALS</td>
<td></td>
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<tr>
<td>Foroozanfar63</td>
<td>1</td>
<td>Cy</td>
<td>BM MUD</td>
<td>no</td>
<td>Yes</td>
<td>3</td>
<td>A + W, eventual rejection</td>
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<tr>
<td>Rappeport64</td>
<td>1</td>
<td>Procarbazine ATS</td>
<td>BM MSD</td>
<td>Acute and chronic</td>
<td>Yes</td>
<td>0.25</td>
<td>Died</td>
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<td></td>
<td></td>
<td>TBI</td>
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<tr>
<td>Hobbs65</td>
<td>1</td>
<td>Cy</td>
<td>BM MUD</td>
<td>no</td>
<td>Yes</td>
<td>7</td>
<td>Rejected at 3 months Died</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pelvic irradiation</td>
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<tr>
<td>Horwitz67</td>
<td>10</td>
<td>Cy, Flu, ATG</td>
<td>PBSC MSD</td>
<td>3 aGvHD (II–IV)</td>
<td>9/10</td>
<td>Median 1.4 (0.66–2.2)</td>
<td>6 A + W 1cGvH 3 died (TRM) (70%)</td>
</tr>
<tr>
<td></td>
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<td>TBI</td>
<td>TCD</td>
<td>2 cGvHD</td>
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<tr>
<td>Seger68</td>
<td>27</td>
<td>Bu, Cy</td>
<td>2 MUD, 9 MSD (all BM)</td>
<td>0</td>
<td>11</td>
<td>Median 2 (0.3–4)</td>
<td>All A + W</td>
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<tr>
<td>Active inflammation</td>
<td>5 Bu, Cy, 1 Bu, Flu; 7 MSD BM</td>
<td>3 aGvHD (II–IV)</td>
<td>7</td>
<td>Median 1.6 (1.2–12)</td>
<td>All A + W, 1 cGvHD resolving</td>
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<tr>
<td>(7)</td>
<td>1 Flu, TBI</td>
<td>1 cGvH</td>
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<tr>
<td>treatment-refractory</td>
<td>5 Bu, Cy, 2 Bu, Flu; 8 MSD BM</td>
<td>3aGvHD (II–IV)</td>
<td>6</td>
<td>Median 1.4 (0.2–5 years)</td>
<td>4 A + W 1 non-engraftment A 4 Died (81%)</td>
<td></td>
<td></td>
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<tr>
<td>infection (9)</td>
<td>1 Bu, Mel; 1 Cy, Flu</td>
<td>2cGvHD</td>
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<tr>
<td>Soncini69</td>
<td>20</td>
<td>9 Bu, Cy, 1 Bu, Mel</td>
<td>10 MSD (9 BM, 1UCBSC)</td>
<td>3 aGvHD (II)</td>
<td>10</td>
<td>Median 6.1 (0.2–9.75)</td>
<td>9 A + W 1 died D + 73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 Bu, Cy, 1 Bu, Flu; 10 MUD (6 BM, 2 PBSC, 2 UCBSC)</td>
<td>2 cGvHD</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>2 Flu, Mel</td>
<td></td>
<td>3 aGvHD (II)</td>
<td>9</td>
<td>Median 1.4 (0.3–5.7)</td>
<td>9 A + W, 1 required 2nd HSCT for non-engraftment 1 died D-1 (aspergillus) (90%)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>1 cGvH</td>
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<tr>
<td>Güngör77</td>
<td>56</td>
<td>Targeted Bu, Flu</td>
<td>21 MFD</td>
<td>5 aGvHD (I)</td>
<td>21</td>
<td>Median 1.9 (0.75–9.75)</td>
<td>20 A + W 1 died after 2nd HSCT for 2e graft failure</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>32 A + W</td>
</tr>
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<td></td>
<td>3 died (1 PTLD, 1 aGvHD, 1 OB) (93%)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Number of patients*</th>
<th>CR</th>
<th>Stem cell source</th>
<th>GvHD</th>
<th>Engrafted</th>
<th>Follow-up (years)</th>
<th>Outcome (OS with cure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morillo-Gutierrez78</td>
<td>70</td>
<td>Treo, Flu; Treo, flu, TT;</td>
<td>36 MUD, 33 PBSC, 1 UCBSC</td>
<td>56 aGvHD (II–IV), 9 cGvHD</td>
<td>64 (6 non-engraftment, 5 re-transplanted)</td>
<td>Median 3-2 (0-45-8-5)</td>
<td>64 A + W 5 died (4 GvHD, 1 sepsis, 1 MOF) (91-4%)</td>
</tr>
<tr>
<td>Khandelwal74</td>
<td>18</td>
<td>14 Bu, Cy</td>
<td>3 MFD, 11 MUD</td>
<td>6 aGvHD (II–III), 4 cGvHD</td>
<td>13</td>
<td>Median 3-8 (1-1–19-4)</td>
<td>11 A + W, 3 died (2 aGvHD, 1 fungal infection) (83%)</td>
</tr>
<tr>
<td>Parta75</td>
<td>40</td>
<td>Bu, alemtuzumab (+TBI for MUD HSCT)</td>
<td>6 MSD PBSC, 34 MUD (4 BM)</td>
<td>18 aGvHD (II–IV), 5 cGvHD</td>
<td>31 (2 non-engraftment, 7 rejection)</td>
<td>Median 3-4 (2–5)</td>
<td>32 A + W 7 died (2 sepsis, 5 MOF) (82-5%)</td>
</tr>
<tr>
<td>Fox85</td>
<td>11</td>
<td>9 Bu, Flu; 2 Flu, Mel</td>
<td>3 MFD, 8 MUD</td>
<td>1 aGvHD (III), 3 cGvHD</td>
<td>11</td>
<td>Median 2-7 (0-6–9-6)</td>
<td>9 A + W 2 died (1 MOF, 1 GvHD) (82%)</td>
</tr>
<tr>
<td>Lum81</td>
<td>55</td>
<td>21 Bu, Cy; 24 Treo, flu (+4 TT)</td>
<td>31 MUD, 4 UCBSC</td>
<td>10 aGvHD (II–IV), 0 cGvHD</td>
<td>54 (7 required boost for falling chimerism)</td>
<td>Median 6-5 (0-32–19-5)</td>
<td>50 A + W 5 died (3 MOF, 1 GvHD, 1 PTLD) (91%)</td>
</tr>
<tr>
<td>Chiesa79</td>
<td>712</td>
<td>113 Bu, Cy; 324 Bu, Flu; 89 Treo, Flu; 58 Treo, Flu, TT</td>
<td>547 MRD/MUD, 134 mMUD</td>
<td>143 aGvHD (II–IV), 123 cGvHD</td>
<td>610 (84 1e or 2e graft failure)</td>
<td>Median 3-75 (1-5–6-9)</td>
<td>620 A + W 92 died (39 sepsis, 30 GvHD, 10 MOF, 13 other) (85-7%)</td>
</tr>
<tr>
<td>Lhomme47</td>
<td>4 (McLeod phenotype)</td>
<td>Bu, Flu</td>
<td>3 MUD, 1 MFD</td>
<td>2 aGvHD (II–III), 0 cGvHD</td>
<td>4</td>
<td>Median 1 (1-7)</td>
<td>3 A + W 1 died (ARDS) (75%)</td>
</tr>
</tbody>
</table>

### Gene therapy
- **Ott90**: 2 Liposomal Bu, Gammaretroviral, 2 MDS, 15% 2 1 A + W post HSCT 1 died – sepsis 2 A + W with no functioning cells 1 died (sepsis)
- **Kang E94**: 3 Bu, Gammaretroviral, Gene silencing, No 0-9 (0-5–2-8)
Today, HSCT for CGD has a high curative rate with low mortality (Table II), although best results are found in young patients with no pre-existing co-morbidities. It is not clear which patients should be offered HSCT. Although residual production of reactive oxygen species, predicted by the specific NADPH oxidase mutation, confers less severe illness and a greater probability of long-term survival, it does not protect against inflammatory sequelae.88

Gene therapy

Given the significant risks associated with earlier transplants, autologous gene addition therapy was developed to improve patient outcomes. Unlike lymphocytes in patients with severe combined immunodeficiencies, genetically-corrected neutrophils demonstrate no survival advantage over faulty neutrophils. As neutrophils survive only a few days in the periphery, long-term production of gene-corrected neutrophils requires engraftment of comparatively high numbers of gene-transduced hematopoietic stem cells, obliging the administration of myeloablative chemotherapy prior to infusion of transduced cells.89 A gamma-retroviral gene therapy trial for CGD performed in Zurich demonstrated significant clinical benefit early post-treatment, with clearance of life-threatening fungal infection90 and restoration of neutrophil extracellular traps.91,92 Clinical benefits did not persist, as functioning neutrophils disappeared due to silencing of gene expression caused by methylation of the LTR promoter elements. Unfortunately, persistent enhancer activity directed transactivation of MDS/EVI proto-oncogenes, causing monosomy 7 and myelodysplasia in three patients, two of whom died, and the third required HSCT. A fourth patient developed clonal expansion with no associated chromosomal abnormalities and was successfully transplanted.93 Other trials using retroviral vectors demonstrated transient clinical benefit only, although no genotoxicity developed.94-96 As with other primary immunodeficiencies, new lentiviral vectors have been developed to reduce the genotoxicity risks associated with retroviral vectors. The first multicentre study to be published details nine high-risk patients, age 2-27 years, with four further patients briefly noted in the discussion.97 All received busulphan AUC of 70-75 mg/l. Eight had an oxidative burst >10% after 12 months; four had >30%, but four had <10% oxidative burst. The minimum oxidative burst required to prevent problems observed in X-linked carriers has to be determined, although autoimmunity appeared not to be associated with a particular %DHR.84 Two patients died of reconstitution-related issues. No genotoxicity events were reported, although maximum follow-up was only 24 months. Nevertheless, these are encouraging results.

Table II. (Continued)

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Number of patients*</th>
<th>CR</th>
<th>Stem cell source</th>
<th>GvHD</th>
<th>Engrafted</th>
<th>Follow-up (years)</th>
<th>Outcome (OS with cure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kang H95</td>
<td>2</td>
<td>Bu, Flu</td>
<td>Gammaretroviral</td>
<td>Gene silencing</td>
<td>No</td>
<td>3</td>
<td>2 A + W with no functioning cells</td>
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<tr>
<td>Grez96</td>
<td>1</td>
<td>Liposomal Bu</td>
<td>Gammaretroviral</td>
<td>MDS</td>
<td>20%</td>
<td>2</td>
<td>Died of sepsis</td>
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<tr>
<td>Kohn97</td>
<td>9</td>
<td>Targeted Bu</td>
<td>Lentiviral</td>
<td>no</td>
<td>Median 31 (1–63)</td>
<td>0-8-3</td>
<td>7 A + W, 1 with loss of function, 2 died (1 pneumonitis, 1 haemorrhage)</td>
</tr>
</tbody>
</table>

ALs, anti-lymphocyte serum; ARDS, acute respiratory distress syndrome; ATS, antithymocyte serum; A + W, alive and well; BM, bone marrow; Bu, busulphan; CR, conditioning regimen; Cy, cyclophosphamide; Fl, fludarabine; GvHD, acute (a) or chronic (c) graft versus host disease; HSCT, haematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; Mel, melphelan; MFD, matched family donor; MSD, matched sibling donor; MOF, multi-organ failure; (m)MUD, (mis)matched unrelated donor; OB, obliterative bronchiolitis; OS, overall survival; PBSC, peripheral blood stem cells; TBI, total body irradiation; TCD, T-cell depleted; Treo, treosulfan; TT, thiopeta; UCBSC, umbilical cord blood stem cells.

*Some patients appear in more than one study.
mRNA and adenoassociated virus 6 delivery of donor constructs. Additionally, transcription activator-like effector nucleases (TALENs) have been used to insert a myelo-specific gp91phox cassette into patient-derived induced pluripotent stem cells (iPSCs). The CRISPR/Cas9 system has also been shown to correct the genetic defect in human cells in X-linked and autosomal recessive CGD.

**Conclusion**

Significant progress in treatment approaches, prognosis, cure and quality of life has been made since the first description of CGD. Even in the early 1990s, there were successful transplant outcomes using matched sibling and matched unrelated donors, and it was concluded that, ‘[i]n selected patients use of a MUD today seems a good option and early transplantation before the establishment of chronic bacterial and fungal infections and organ damage would lessen the risk of reaction and decrease the severity of GvHD in the post-graft period’, (p. 809). Certainly today, patients diagnosed with CGD should be considered early on for curative stem cell therapy. The role of gene therapy over HSCT for X-linked CGD should be considered early on for curative stem cell therapy. The role of gene therapy over HSCT for X-linked CGD. The CRISPR/Cas9 system has also been used to insert a myelo-specific gp91phox cassette into patient-derived induced pluripotent stem cells (iPSCs). The CRISPR/Cas9 system has also been shown to correct the genetic defect in human cells in X-linked and autosomal recessive CGD.

**References**


