Clinical Reviews in Allergy & Immunology (2021) 61:101–113
https://doi.org/10.1007/s12016-020-08800-x

Chronic Granulomatous Disease: a Comprehensive Review

Hsin-Hui Yu[1] · Yao-Hsu Yang[1] · Bor-Luen Chiang[2]

Published online: 10 June 2020

Abstract

Chronic granulomatous disease (CGD) is a primary immunodeficiency of phagocyte function due to defective NADPH oxidase (phox). Compared with the common types of *CYBB*/gp91^{phox}, *NCF1*/p47^{phox}, and *CYBA*/p22^{phox} deficiency, *NCF4*/p40^{phox} deficiency is a mild and atypical form of CGD without invasive bacterial or fungal infections. It can be diagnosed using serum-opsonized *E.coli* as a stimulus in dihydrorhodamine (DHR) assay. Patients with *CYBC1*/Eros deficiency, a new and rare form of CGD, present as loss of respiratory burst and gp91^{phox} expression in phagocytes. Neutrophils from patients with CGD are deficient in neutrophil extracellular traps (NETosis), autophagy, and apoptosis. The hyper-activation of NF-κB and inflammasome in CGD phagocytes also lead to long-lasting production of pro-inflammatory cytokines and inflammatory manifestations, such as granuloma formation and inflammatory bowel disease-like colitis. Patients with CGD and X-linked female carriers also have a higher incidence of autoimmune diseases. The implementation of antimicrobial, anti-fungal, and interferon-γ prophylaxis has greatly improved overall survival. Residual NADPH oxidase activity is significantly associated with disease severity and the chance of survival of the patient. New therapeutic approaches using immunomodulators for CGD-related inflammatory manifestations are under investigation, including pioglitazone, tamoxifen, and rapamycin. Hematopoietic stem cell transplantation (HSCT) is the curative treatment. Outcomes of HSCT have improved substantially over the last decade with overall survival more than 84–90%, but there are debates about designing optimal conditioning protocols using myeloablative or reduced-intensity regimens. The gene therapy for X-linked CGD using hematopoietic stem and progenitor cells transduced ex vivo by lentiviral vector encoding the human gp91phox gene demonstrated persistence of adequate oxidase-positive neutrophils in a small number of patients. Gene therapy using genome-editing technology such as CRISPR/Cas9 nucleases is a promising approach for patients with CGD in the future.

Keywords Chronic granulomatous disease · Prophylaxis · Interferon-γ · Transplantation · Gene therapy

Introduction

Chronic granulomatous disease (CGD) was initially called "fatal granulomatous disease of childhood" in the 1950s [1]. It is a rare primary immunodeficiency disease characterized by recurrent life-threatening bacterial, fungal infections, and tissue granuloma formation, which is caused by defects in genes encoding the subunits of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex [2]. The incidence of CGD is estimated at approximately 1/200,000 live births in the United States, and the highest incidence estimated to be 1.5/100,000 in the Israel Arab population [3–5].

Pathogenesis

The NADH oxidase is a multicomponent enzyme consisting of a membrane-bound, heterodimeric b-type cytochrome comprising the subunits gp91^{phox} (also known as Nox2) and p22^{phox}, together with cytoplasmic subunits p47^{phox}, p40^{phox}, and p67^{phox}. Upon activation, the cytosolic components translocate to the membrane and associate with a cofactor Rac2 GTPase. In a recent study, essential for reactive oxygen species (Eros) transmembrane protein acts as a chaperone and is

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12016-020-08800-x) contains supplementary material, which is available to authorized users.

✉ Bor-Luen Chiang
gicmbor@ntu.edu.tw

[1] Department of Pediatrics, National Taiwan University Children's Hospital, Taipei, Taiwan

[2] Department of Medical Research, National Taiwan University Hospital, Taipei, Taiwan

required for the gp91^{phox} and p22^{phox} heterodimer expression [6, 7] (Fig. 1).

The NADPH oxidase transfers electrons to molecular oxygen to generate superoxide anion (O_2^-), which is dismutated to hydrogen peroxide (H_2O_2). These two primary reactive oxygen species (ROS) can be further transformed into more reactive metabolites, such as hydroxyl radical ($OH^{\cdot}$) or hypochlorous acid (HOCl). HOCl is highly microbicidal, and its formation requires neutrophil myeloperoxidase (MPO) [8]. ROS are chemically reactive metabolites of oxygen. The phagocyte NADPH oxidase generates higher levels of ROS than other cellular oxidases such as mitochondria or nitric oxide synthases.

Phagocytes NADPH oxidase can be assembled in the plasma membrane and membranes of intracellular vesicles. ROS production in phagocytes, which is also known as a "respiratory burst" reaction, contributes to microbial killing and triggering the formation of neutrophil extracellular traps (NETs) [9]. NETs are decondensed chromatin fibrils coated with granular proteases and histones, which can trap and kill extracellular pathogens. Neutrophils from patients with CGD are deficient in NETosis in previous reports [10, 11].

Mice defective for ROS and patients with CGD have a proinflammatory phenotype. Nox2 expressed in neutrophils and macrophages limits NF-κB activation and proinflammatory cytokines production [12]. NADPH oxidase deficient phagocytes from patients with CGD display a defect in autophagy and activation of inflammasome [13–15], which leads to long-lasting production of proinflammatory cytokines (TNF-α, IL-1β, IL-6), interferons, chronic hyper-inflammation, and autoimmunity [14, 16]. The increased L-1β levels by activated macrophages provide a signal for the massive influx of neutrophils in the granulomatous lesions. Rescue of a functional NADPH oxidase complex *Ncf1*/p47^{phox} in mononuclear phagocytes (macrophages and dendritic cells), not in neutrophils, leads to the loss of the hyper-inflammation in a transgenic mice model of CGD [16]. Recent studies also demonstrated the crucial roles of NADPH oxidase in limiting systemic inflammation and immunomodulation in innate and adaptive immune system [12].

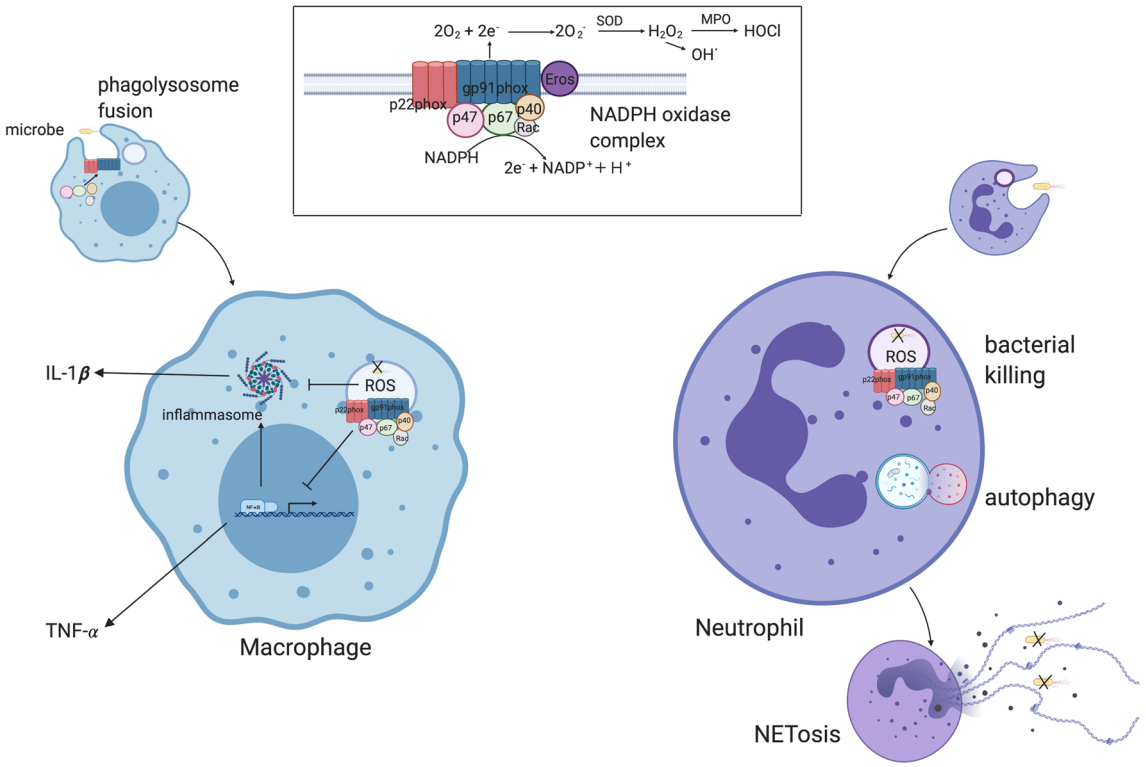

Fig. 1 Structure and function of the NADPH oxidase. NADPH oxidase consists of gp91phox, p22phox in the plasma membrane and intracellular granules. After phagocytes activation, the cytosolic components p47^{phox}, p40^{phox}, p67^{phox} and the GTPase protein Rac2 are translocated to the membrane of phagosome, where they assemble with p22phox and gp91phox to form a functional NADHP oxidase. Eos, a chaperone protein, is required for the gp91^{phox} and p22^{phox} heterodimer expression. The NADPH oxidase catalyze the formation of superoxide free radical (O_2^-) by transferring an electron from NADPH to molecular oxygen (O_2). The superoxide is converted to hydrogen peroxide (H_2O_2) spontaneously or by superoxide dismutase (SOD). Hydrogen peroxide is converted to more potent reactive oxidants, such as $OH^{\cdot}$ or HOCl by myeloperoxidase (MPO) for bacterial killing. The activation of NADPH oxidase suppresses NF-κB activity, the following nuclear transcription of proinflammatory cytokines, and the inflammasome-mediated IL-1β production in macrophages. NADPH oxidase enhances autophagy and neutrophil extracellular traps activation and release (NETosis) in stimulated neutrophils

The genetic basis for CGD is summarized in Table 1. Germline null mutations in *CYBB*, the gene encoding the gp91 subunit of the NADPH oxidase, impair the respiratory burst of all types of phagocytes and result in X-linked CGD. Over 90% of patients with *NCF1* gene mutations in the $p47^{phox}$ subunit of the oxidase complex carry the deletion c.75_76delGT (ΔGT) [2, 17, 18]. Patients with Eros deficiency due to *CYBC1* homozygous mutation presented as infectious and inflammatory symptoms due to loss of respiratory burst and $gp91^{phox}$ expression in phagocytes [6].

Diagnosis

According to European Society for Immunodeficiencies (ESID) working definitions for the clinical diagnosis of inborn errors of immunity, patients with at least one of the following need work-up for CGD: (1) deep-seated infection due to bacteria and/or fungi (abscesses, osteomyelitis, lymphadenitis), (2) recurrent pneumonia, (3) lymphadenopathy and/or hepatomegaly and/or splenomegaly, (4) obstructing/diffuse granulomata in gastrointestinal or urogenital tract, (5) chronic inflammatory manifestations (colitis, liver abscess, and fistula formation); (6) failure to thrive, (7) affected family member [19].

The nitroblue tetrazolium (NBT) test has been used historically to measure superoxide generation in phagocytes after phorbol myristate acetate (PMA) stimulation. The yellow-colored dye is reduced by the NADPH oxidase complex in neutrophils to formazan, which is a dark-blue precipitate in the stimulated phagocytes [20]. Since the late 1990s, the activity of oxidase activation can be measured by flow cytometry using fluorescent probes 2′7′-dichlorofluorescein diacetate (DCF) or dihydrorhodamine (DHR)-123. In stimulated neutrophils, DHR-123 is oxidized by hydrogen peroxide (produced in the presence of normal NADPH oxidase and myeloperoxidase) to rhodamine-123 that emits fluorescence [21]. Frequently used stimuli are PMA, serum-treated zymosan and the bacterial peptide formyl-methionyl-leucyl-phenylalanine (fMLP) [22]. The diagnosis of CGD can be made by the absent or significantly decreased respiratory burst in stimulated neutrophils by NBT, DHR, or DCF test at least twice [19, 21].

The mean fluorescence intensity of DHR test has been shown to be correlated with reactive oxygen intermediate production and survival in CGD patients. Compared with absent superoxide production levels in $gp91^{phox}$ deficient patients, $p47^{phox}$ deficient patients were associated with decreased (not absent) ROS production and had better survival [23]. The PMA-induced DHR oxidation assay can differentiate between X-linked CGD, autosomal recessive (AR)-CGD, and X-linked carrier status [21]. However, routine DHR assay could be normal or mildly impaired in patients with *NCF4*/$p40^{phox}$ deficiency. Using serum-opsonized *E.coli* as a stimulus in DHR tests to diagnose *NCF4*/$p40^{phox}$ deficiency is recommended [24].

Abnormal DHR neutrophil assay results should be confirmed with gene mutation analysis, which is also mandatory for genetic counseling. Genetic and prenatal testing can be useful for screening for other family member carriers. Besides, large deletions in Xp21.1 have been reported in some patients with X-linked CGD. A chromosome microarray analysis is indicated for patients suspected as X-linked CGD but with clinical manifestations of other genetic defects that lie in close proximity to *CYBB* at Xp21.1, such as McLeod syndrome (Duchenne muscular dystrophy, retinitis pigmentosa, or ornithine transcarbamylase deficiency) [25].

Differential Diagnosis

Hypomorphic (p.Thr178Pro, p.Gln231Pro) missense mutations of *CYBB* gene cause defective respiratory bursts in macrophages and B cells (not in neutrophils or monocytes) and X-linked recessive Mendelian susceptibility to mycobacterial disease (MSMD) [26]. *RAC2* deficiency results in decreased neutrophil chemotaxis, ROS generation, and neutrophil immunodeficiency syndrome [27]. Myeloperoxidase (MPO)

Table 1 Genetic defects of chronic granulomatous disease (CGD)

Protein (subunit)		Gene	Locus	OMIM	inheritance	Frequency (%)
NADPH oxidase	$gp91^{phox}$ (cytochrome $B_{558\beta}$)	*CYBB*	Xp21.1	306400	X-linked	65
	$p22^{phox}$ (cytochrome $B_{558\beta}$)	*CYBA*	16q24.2	608508	AR	5–10
	$p47^{phox}$	*NCF1*	7q11.23	608512	AR	25
	$p67^{phox}$	*NCF2*	1q25.3	608515	AR	5–10
	$p40^{phox}$	*NCF4*	22q12.3	613960	AR	Rare
Cytochrome B-245 Chaperone 1 (Eros)		*CYBC1*	17q25.3	618334	AR	Rare

Abbreviation: Nicotinamide adenine dinucleotide phosphate, NADPH; Essential for reactive oxygen species, Eros; autosomal recessive, AR

deficiency has defects in the oxidation of hydrogen peroxide to hypohalous acid in phagocytes. Patients frequently are asymptomatic or otherwise are susceptible to *Candida* infections. Complete MPO deficiency exhibit low DHR signals and normal NBT test results [28]. A rare, severe form of glucose-6-phosphate dehydrogenase (G6PD) deficiency lacks sufficient NADPH generation for the NADPH oxidase in the leukocytes, thus similar to a mild type of CGD [29].

Clinical Presentations

Most patients with CGD present initially with infections early in life and are diagnosed before five years old. Patients with X-linked CGD tend to present with earlier onset and more severe disease than patients with AR-CGD [30]. AR-CGD can present in adulthood in which residual production of superoxide can be seen [21]. ROS production mainly mediates the effect of genotype on phenotype and has been directly associated with survival [23]. *NCF4*/p40^{phox} deficiency is considered as a mild, atypical form of CGD that patients suffer from hyper-inflammation and peripheral infections (mostly *Staphylococcal*), but do not have invasive bacterial or fungal infections commonly seen in X-linked CGD [24].

Infection

Patients have increased susceptibility to infections by catalase-positive organisms, including *Staphylococcus aureus, Burkholderia cepecia* complex*, Serratia marcescens, Nocardia* species*, and Aspergillus* species in the lungs, lymph nodes, skin, liver, and bones [30, 31]. Invasive fungal infections remain the most common cause of mortality in CGD, including *Aspergillus* species, *Phaeoacremonium parasiticum*, and *Candida* species [30]. Aspergillus spp. (most commonly *Aspergillus fumigatus* or *A. nidulans*) account for 35% of all deaths attributable to infections in patients with CGD, which often presented as pneumonia or pneumonia with dissemination to the liver or brain [3]. Tuberculosis, *Mycobacterium bovis* Bacille Calmette-Guérin (BCG), or *Salmonella* species are also important causes of infections in regions of the world where these organisms are prevalent, or BCG vaccine is routinely administered at birth [32]. Rarely, *Actinomyces* species (catalase-negative organisms) infection, or methylotrophs (*Granulibacter bethesdensis, Acidomonas methanolica, Methylobacterium lusitanum)* necrotizing lymphadenitis or fatal meningitis also have been reported [33, 34]. Common and uncommon organisms found in patients with CGD are summarized in Table 2 and Supplementary Table 1 [30, 32, 33, 35–37]. The application of genomic or 16S rRNA sequencing and molecular probes to target tissues can identify the microorganisms that are difficult to culture, such as Phellinus or methylotroph infections.

Inflammation and Autoimmunity

Dysregulation of pro-inflammatory cascades and defective apoptosis predispose patients to granulomatous inflammation, typically in the lungs, gastrointestinal and genitourinary tracts [17, 38]. Nearly 50% of patients encounter inflammatory bowel disease (IBD), which resembles Crohn's disease [39]. Chronic complications, including growth failure, decreased pulmonary function, poor quality of life are common in patients who have not undergone a transplant [40, 41].

A high incidence of infections, accompanied by an inappropriate hyperinflammatory response may render these patients with CGD at risk of developing hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome (MAS). There were 29 CGD patients with *CYBB, CYBA, NCF1, and CYBC1* mutations developed HLH/MAS in the previous literature [6, 32, 42–44].

Treatment

Acute Infections

Symptoms of infections are initially vague. A significant and sustained rise of laboratory markers of inflammation, such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), often indicates an occult infection that requires imaging (computed tomography or magnetic resonance imaging), microbiologic diagnosis, or tissue biopsy. Prolonged use of antibiotics/anti-fungal treatment is needed even for common bacterial or fungal infections. Voriconazole or posaconazole may empirically use if mold infection is suspected [31]. Granulocyte transfusions is safe to use in the setting of invasive fungal infections, but they could lead to alloimmunization and complicate future stem cell transplantation [45, 46]. The overall incidence and severity of infections decreased in the 2nd decade of life, but the increased risk of infections is lifelong [30].

Prophylactic Antibiotic and Anti-Fungal Therapy

Trimethoprim-sulfamethoxazole (TMP/SMX) and itraconazole prophylaxis is the cornerstone of prevention of bacterial and fungal infections [30, 47]. Prophylaxis with TMP/SMX prolonged life-threatening infection-free intervals from 1 episode every ten months to 1 episode every 40 months in patients with CGD [48]. TMP/SMX prophylaxis also decreased the incidence of bacterial infections by 56% (from 15.8 to 6.9 infections per 100 patient-months) in patients with X-linked CGD and 66% (from 7.1 to 2.4 infections per 100 patient-months) in patients with AR-CGD [49]. In patients with sulfa allergy, TMP alone can be used as an alternative treatment. In patients with TMP/SMX intolerance, other options, including

Table 2 Common organisms and treatment of infections in CGD

Category	Organism	Presentations of infection								Treatment
		Soft tissue	LN	liver abscess	Bone	Lung	sepsis	Brain	GI	
Bacteria	*Staphylococcus aureus*	+	+	+	+	+	+			oxacillin, vancomycin, linezolid, teicoplanin, daptomycin, ceftaroline
	Burkholderia spp. • *B. cepacia, B. gladioli, B. pseudomallei*		±			+	+			TMP-SMX, meropenem, ciprofloxacin
	Serratia marcescens	+	+		+	+	+			Piperacillin-tazobactam, ciprofloxacin, carbapenem
	Nocardia spp. • *N. asteroids, N. nova, N. otitidiscaviarum, N. farcinica*		±		+	+		+		TMP-SMX, imipenem, linezolid
	Klebsiella spp., E. coli	+				+	+		+	cephalosporins, fluoroquinolone, meropenem, imipenem
	Pseudomonas spp.	+				+	+		+	Piperacillin-tazobactam, cefepime, meropenem, imipenem
	Salmonella spp.	+		+	+		+		+	ciprofloxacin, ceftriaxone, cefixime
Fungi	*Aspergillus spp.* • *A. fumigatus, A. nidulans, A. viridinutans, A. flavus, A. terreus, A. niger*		+		+	+		+		voriconazole, liposomal amphotericin B, posaconazole, caspofungin, micafungin
Mycobacteria	*Mycobacterium tuberculosis*	+	+	+	+	+		+	+	Isoniazid, rifampin, pyrazinamide, ethambutol
	BCG	+	+	+	+	+		+	+	Isoniazid

Abbreviations: lymphadenitis, LN; Mycobacterium bovis Bacillus Calmette–Guérin, BCG; gastrointestinal, GI; trimethoprim-sulfamethoxazole, TMP-SMX

fluoroquinolone, second- or third-generation cephalosporin or clindamycin can be considered [47].

Prophylaxis with itraconazole has shown to be effective in reducing the frequency of fungal infections and well-tolerated in patients with CGD [50]. It also prolonged the median time to invasive fungal infection from 4 years to 10 years in patients with CGD and decreased mortality rate [51]. Because of the emergence of itraconazole resistant organisms and occasional medication intolerance, the use of posaconazole or voriconazole as secondary prophylaxis has increased [47]. In recent studies in patients with X-linked CGD under azole prophylaxis, *Phellinus* species emerge as causes of indolent but refractory fungal infections. Sending tissue specimens for molecular diagnosis is essential in managing non-*Aspergillus* invasive fungal infections [37].

Interferon-Gamma (IFN-γ) Prophylaxis

Immunomodulatory therapy with IFN-γ is part of the prophylactic regimen in many centers, while some experts use IFN-γ only in the setting of acute infection, rather than as primary prophylaxis [35]. A randomized, double-blind placebo-controlled study showed that IFN-γ 50 μm/m^2 3 times per week significantly reduced the number of hospitalizations and serious infections (22% treatment vs. 46% placebo group) after a follow-up period of 8.9 months [52]. However, a prospective study comparing prophylaxis with TMP/SMX and itraconazole versus addition of IFN-γ showed no significant difference in the rates of infections [53].

IFN-γ therapy can increase nitric oxide (NO) production by neutrophils and circulating NO levels in patients with CGD. NO acts as a bactericidal agent and plays a role in host defense [54]. In a recent study, IFN-γ has been documented to restore autophagy for *Aspergillus* through death-associated protein kinase 1 (DAPK1) pathway and inhibit inflammasome activation in mouse models and patients with CGD [55].

Common adverse effects of IFN-γ are flu-like symptoms (fever, fatigue, myalgia), rash, and local injection-site reactions (erythema or tenderness). Many experts consider the risk/benefit ratio to be favorable for the use of IFN-γ to prevent invasive fungal infections, particularly in young patients with X-linked CGD and those with a history of invasive fungal infections [35].

Inflammatory Complications

Corticosteroids

Corticosteroids may be useful for antral and urethral obstruction, severe granulomatous colitis, lung granuloma, and liver abscess. Liver abscesses occur in about one-third of patients and are commonly caused by *S. aureus*. Liver abscesses in CGD are frequently multi-loculated with a thickened pseudocapsule. Under antibiotics treatment, surgical resection of liver abscesses is considered to be safe and associated with less recurrence and shortened hospitalization in patients with CGD [56]. Treatment with CGD-associated liver abscesses with corticosteroids (median dose 1 mg/kg/day and median duration of five months) and targeted antimicrobial therapy was associated with improved outcome and fewer subsequent hepatic interventions, compared to invasive treatments such as interventional radiology therapy or open liver surgery [57]. Steroids might reduce systemic inflammation, restore local immunity in the liver, and provide better tissue penetration of the antibiotics in a less inflamed environment [57].

Immunomodulators and Biological Therapies

The treatment of hyper-inflammation remains a challenge because immunomodulators can be associated with a higher risk of infections in CGD. The use of TNF-α antagonist infliximab has shown to be effective in treating CGD associated colitis, but it predisposes patients to severe infections, including deaths [58]. Extreme caution should be taken, such as aggressive antimicrobial prophylaxis and aggressive surveillance for infections under anti-TNF treatment. Clinical trials of IL-1β receptor antagonist anakinra in patients with CGD colitis have mixed results [59, 60]. Inhibition of mammalian target of rapamycin complex 1 (mTORC1) by rapamycin has been shown to inhibit inflammasome activation, IL-1β and TNF-α production of phagocytes in vitro through autophagy induction and immunomodulatory effect in phagocytes from patients with CGD [61]. However, the clinical effectiveness of rapamycin in the management of CGD related inflammatory complications remains to be assessed by clinical trials.

Pioglitazone or rosiglitazone, a peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist, has been approved for the treatment of type 2 diabetes. It has been shown that pioglitazone restores phagocyte (monocytes and neutrophils) mitochondrial superoxide production, suppresses acute sterile inflammation (peritonitis), and enhances bactericidal capacity in vitro and in vivo in mice models with X-CGD [62–64]. The clinical improvement by partially restored inflammatory status and host defense was observed in a 5-month-old infant with X-linked CGD with multiple severe infections who received pioglitazone. After titration of pioglitazone to 3 mg/kg, a DHR assay showed oxidative activity of 12.9% of granulocytes (versus total absence respiratory burst before therapy) [65].

Pioglitazone and rosiglitazone can also induce significant NET formation in CGD patients [63]. Although the exact mechanism for induction of NETosis through mitochondrial ROS enhancement is unclear, it has been shown to depend on calcium-activated potassium channel and signaling cascades, including AKT and p38 [66]. In a recent study, Tamoxifen, an FDA-approved drug in use for breast cancer treatment, has been shown to restore NETosis and NET-mediated bacterial killing in a ROS-independent manner by activating autophagy in neutrophils from patients with CGD [11].

Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation (HSCT) can be curative for CGD. However, high-risk patients with intractable infection or autoinflammation as well as adolescents and young adults have traditionally been difficult cases for HSCT due to higher rates of organ dysfunction, graft failure, graft-versus-host disease (GVHD) and transplant-related mortality. Since levels of residual superoxide production have correlated well with overall survival, HSCT has been recommended in patients with X-linked or autosomal recessive CGD if a matched donor is identified, and if no residual oxidase activity or if severe disease complications develop [67]. The use of non-myeloablative conditioning regimens has greatly decreased the regimen related toxicity and improved survival (Table 3) [40].

It is now expected that about 90% of patients with CGD will survive into young adulthood with or without HSCT given an improved diagnosis, prophylaxis with interferon-γ, itraconazole, and antimicrobials agents [40]. However, older children and adolescents are especially noncompliant with prophylaxis therapy, increasing their risk of complications. The overall quality of life is reduced in patients with CGD, whereas patients with CGD who have undergone HSCT report quality of life comparable to healthy children [68]. The ESID and the European Blood and Marrow Transplantation (EBMT) indications for HSCT in patients with X-linked or AR-CGD include patients with a matched donor or mismatched unrelated donor plus one clinical or social complications (Table 4).

Finding a suitable donor is one of the most important steps for patients who undergo allogeneic HSCT. The ideal donor for HSCT is HLA-matched sibling donors (MSD) with the highest overall survival. But based on average family size, the availability of such a donor is less than 30% of patients [69, 70]. HLA-matched unrelated donors (MUD) have been preferred in the absence of MSD. Alternative donors such as HLA-mismatched unrelated donors (MMURD with one or more HLA mismatch at the HLA-A, HLA-B, HLA-C, or HLA-DRB1 loci), HLA-haploidentical donors, and umbilical cord blood (UCB) are considered tertiary options. Greater risk of graft failure, graft versus host disease (GVHD), and transplant-related mortality with grafts

Table 3 Summary of the outcomes of hematopoietic stem cell transplantations in patients with CGD

Year	N	Age (yrs)	XL (%)	Regimen	Donor type	Donor (%)	OS (%)	EFS (%)	FU (yrs)	Ref
2013	14	10.7	76.9	Bu/Cy or Flu/ATG or A	MRD/MUD/MMUD	>92 (60–100)	92.9	78.6	7.7	[93]
2014	56	12.7	60.7	RIC: Flu/ATG or A/Bu	MRD/MUD	≧90	93	89	1.75	[78]
2016	70	8.9	80	RTC: Treo/Flu or Cy/TT or ATG or A or TBI	MRD*/MUD/MMUD	95	91	81	2.8	[75]
2016	4	14	75	RIC: A/Flu/Mel	MUD/ 7/8 MMUD	70 (11–100)	100	50	1.65	[94]
	14	3.18	36	MAC: Bu/Cy/ATG	MUD/ 7/8 MMUD	>95	78.6	78.6	5.27	
2019	7	15	85.7	RTC: Bu or Cy or TT/Flu/ATG, TBI	MRD/MUD	>92	100	85.7	2.7	[76]
2019	50	13.1	72	RIC for non-UCB, MAC for UCB	MRD/MUD/MMUD/haploidentical	>90	88	82	1.54	[77]
2019	145	7	73.1	RIC/RTC: Bu/Flu, Bu/TBI, Flu/Mel; MAC: Bu/Cy	MRD/MMRD/MUD/MMUD	>90 (0–100)	84.1	82.1	3.5	[81]

Abbreviations: RIC, reduced-intensity conditioning; RTC, reduced-toxicity conditioning; CGD, chronic granulomatous disease; EFS, event-free survival; OS, overall survival, MRD, HLA-matched related donor; MMRD, HLA-mismatched related donor; MUD, HLA-matched unrelated donor; MMUD, HLA-mismatched unrelated donor; Flu, fludarabine; Mel, melphalan; Treo, treosulfan; A, Alemtuzumab (anti-CD52); Bu, busulfan; Cy, cyclophosphamide; ATG, antithymocyte globulin; TT, thiotepa; TBI, total body irradiation; UCB, umbilical cord blood

Age: median age at transplantation; FU (yrs): follow-up median duration post transplantation (years); Donor (%), donor chimerism; XL, X-linked

*includes one patient received a $CD3^+$ $TCR\alpha\beta^+/CD19^+$ depleted haploidentical parental transplant

from genetically distant donors drives the donor hierarchy [71, 72]. However, advances in HLA typing technology, GVHD prophylaxis, and management of complications have substantially reduced the risk of mortality, and HSCT from an alternative donor has been increasingly safe and feasible [69, 72]. The outcomes of haploidentical transplantation can differ largely according to GVHD prophylaxis methods which include T cell depletion, post-transplant cyclophosphamide, and anti-thymocyte globulin [72–74]. The average success rate of recent studies of HSCT for CGD was 40/42 (95.2%) for MSD, 97/107 (90.6%) for MUD, 20/23 (87.0%) for MMUD, 3/3 (100%) for haploidentical donors, and 4/5 (80%) for UCB. The complication rate of HSCT was 50.0% for MSD, 57.9% for MUD, 78.3% for MMUD, 33.3% for haploidentical donor, and 20% for UCB [75–78].

From the studies of X-linked carrier females, it suggests that the level of myeloid chimerism that protects patients from infection may be lower than that to protect from autoinflammation [79, 80]. In recent studies, reduced-intensity/reduced toxicity or myeloablative conditioning regimens are safe and effective in CGD patients, including patients with IBD. The results achieved more than 90% myeloid donor chimerism and resolution of inflammatory colitis by two years following allogeneic HSCT [76, 81]. The study by Güngör et al. in 2014 also demonstrated that improvement in pulmonary function after HSCT in adults [78].

A recent study of 507 CGD patients by Yonkof et al. and the United States Immunodeficiency Network reported that transplant-related survival improved in patients who underwent allogeneic HSCT at age 14 years or younger (93% vs. 82% at five years post-transplant). Overall survival (OS) was negatively associated with *CYBB* mutation (HR 6.25, $p = 0.034$). HSCT was associated with reduced infection incidence and improved functional performance. The survival was estimated to be 65% 30 years after diagnosis [77].

Table 4 ESID/EBMT guidelines 2017 for hematopoietic stem cell transplantations in patients with X-linked or AR-chronic granulomatous disease

Indications

- Non-availability of specialist medical care
- Non-compliance with long-term antibiotics/antimycotic prophylaxis
- ≧1 life-threatening infection in the past
- Severe granulomatous disease with progressive organ dysfunction (e.g. lung restriction)
- Steroid-dependent granulomatous disease (e.g. colitis)
- Ongoing therapy-refractory infection (e.g. Aspergillosis)
- After emergence of premalignant clones or MDS (e.g. after gene therapy)

Protocols

- Myeloablative conditioning: for umbilical cord blood or haploidentical donor
- Reduced intensity conditioning: for MSD, MUD, MMUD

Abbreviations: AR, autosomal recessive; MSD, HLA-matched sibling donor; MUD, HLA-matched unrelated donor; MMUD, HLA-mismatched unrelated donor; MDS, myelodysplastic syndrome

Adopted from EBMT website: https://www.ebmt.org/ebmt/documents/esid-ebmt-hsct-guidelines-2017

Gene Therapy

The clinical trials of hematopoietic stem and progenitor cell gene therapy were initiated with gammaretroviral vectors. After the occurrence of insertional oncogenesis following

gammaretroviral-based gene therapy, the development of gene therapy shifted away from gamma-retrovirus to lentiviral vectors since 2000 [82, 83]. From the previous studies of female carriers of X-CGD, it is estimated that more than 10–20% oxidase-positive circulating myeloid cells corrected by gene therapy may reach clinical benefit with normal resistance to infection [84].

To retain the efficacy, minimize the mutagenic risk, and enhance safety, a self-inactivating lentiviral vector was designed with a chimeric internal promotor that preferentially drives $gp91^{phox}$ expression in phagocytes. The gene therapy for X-linked CGD using autologous $CD34^+$ enriched cell population that contains hematopoietic stem and progenitor cells (HSPCs) transduced ex vivo by this lentiviral vector encoding the human gp91phox gene demonstrated stable vector copy numbers (0.4 to 1.8 copies per neutrophil) and the persistence of 16% to 46% oxidase-positive neutrophils in six of the nine patients at 12 months follow-up [84]. Based on this promising result, the U.S. Food and Drug Administration (FDA) has granted this gene therapy OTL-102 orphan drug designation for the treatment of X-linked CGD in January 2020.

Despite the advances in the vector design, gene therapy based on the integration of an additional copy of a wild-type gene carries unavoidable risks of insertional mutagenesis. New genome editing technologies can mediate gene addition, gene ablation, gene correction, and other highly targeted genome modifications on cells ex vivo or in vivo. Gene editing via site-specific endonucleases such as zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), or CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9 nucleases has great potential as a therapeutic approach for monogenic hematological diseases because of the availability of autologous HSPCs. A targeted DNA alteration is initiated by the creation of a nuclease-induced double-stranded break (DSB). ZFN or TALEN approaches require the design of a specific pair of nucleases for each new DNA target [85, 86]. CRISPR/Cas9 nucleases, guided by a designed single-guide RNA complementary to the target site of interest, can achieve efficient DSB, which undergo repair by non-homologous end joining (NHEJ) causing insertions or deletions or by homology-directed repair (HDR) allowing donor DNA incorporation [87]. Correction of mutations at a single-base level without DSB via Cas9-based targeting of "base editors" has been reported [88].

Preclinical studies using targeted gene-editing technology for gene targeting with HDR in induced pluripotent stem cells (iPSCs) or HSPCs from patients with CGD have achieved a functional restoration of expression of NADPH oxidase genes and NADPH oxidase activity in differentiated neutrophils (Table 5). The major advantages of iPSCs are that they represent an unlimited source of stem cells. The corrected iPSCs-derived granulocytes and macrophages provide sufficient ROS levels, induction of NET formation, and the ability to kill bacteria [89]. There are many challenges of the field to overcome, including genotoxicity from off-target genome editing, increasing gene editing efficiency to levels necessary for effective treatment, immune responses to the nucleases, and the high price. These will require further studies to reach the goal of clinical applications.

X-Linked Carriers of CGD

X-linked CGD carrier females usually show random X chromosome inactivation by lyonization that one X chromosome is silenced epigenetically. X-linked carrier status can be demonstrated by DHR tests of the two populations of neutrophils. Those neutrophils with inactivation of the *CYBB* mutated X chromosome will have a normal superoxide production, whereas those neutrophils with inactivation of the normal X chromosome will have a defective respiratory burst [90].

The female carriers of X-linked CGD usually do no develop significant infections. However, infection risk is increased in

Table 5 Target repairs using gene editing technology for patients with CGD

Year	Target gene	Tools	Edited cells	Effects in corrected phagocytes or in vivo	Ref
2011	*CYBB*	ZFNs	iPSCs	Restore gp91 expression and NADPH oxidase activity in vitro	[95]
2015	*CYBB*	CRISPR/Cas9	iPSCs	Restore NADPH oxidase activity in vitro	[96]
2015	*CYB, NCF1, NCF2, CYBA, NCF4*	ZFNs	iPSCs	Restore NADPH oxidase activity (16–91.5% DHR^+), bacterial killing in vitro	[97]
2015	*CYBB*	TALEN	iPSCs	Restore NADPH oxidase activity in vitro	[98]
2016	*CYBB*	ZFNs	HSPCs	Donor template insertion (38%) in vitro and in 4–11% xenograft in mice	[99]
2017	*CYBB,* c.C676T	CRISPR/Cas9	HSPCs	Restore gp91 expression and NADPH oxidase activity (19.3% DHR^+) in vitro, 14% xenograft in mice	[100]
2017	*CYBB*	TALEN or CRISR/Cas9	iPSCs	Restored $gp91^{phox}$and ROS activity in vitro	[101]
2019	*NCF1,* c.75_76delGT	CRISPR/Cas9	iPSCs	Restore NADPH oxidase activity (99% DHR^+) and bacterial killing in vitro	[89]

Abbreviations: iPSCs, induced pluripotent stem cells; HSPCs, hematopoietic stem and progenitor cells; DHR, dihydrorhodamine-123 test

those carrier females who have a normal neutrophil population of less than 20% by the study by Marciano BE et al. [79]. Skewing (non-random) of X chromosome inactivation occurs in a minority (22%) of female carriers. The X-linked CGD carrier females with CGD-type infection had median %DHR$^+$ values of 8% (range 0.06% to 48%), and those carriers with autoimmune or inflammation had median %DHR$^+$ values of 39% (range 7.4% to 74%) [79]. About half of X-linked female carriers have various symptoms of autoimmunity, such as Raynaud phenomenon, arthritis, discoid lupus, aphthous ulcers, granulomatous colitis, systemic lupus erythematosus (SLE), and hypothyroidism/hyperthyroidism [79, 80, 91].

X-linked carrier females need therapy for infections or autoimmunity when required. Antimicrobial and antifungal prophylaxis should be considered for cases with recurrent infections and low DHR responses and cases under immunosuppression or biologic treatments for autoimmunity [90]. Allogeneic HSCT showed successful treatment for refractory IBD in two X-linked female carriers [92].

Summary

The development of effective infection prophylaxis regimens and aggressive interventions have much improved the prognosis in the past decade. It is important to optimize conditioning regimens of HSCT to achieve safety and a higher level of donor chimerism necessary to protect against infections and inflammatory complications. Gene therapy is a promising approach for patients with CGD. More studies are needed to evaluate long-term outcomes of contemporary and experimental CGD management, including gene therapy and other potential targeted therapy.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Research with Animals and Human Subjects This article does not contain any studies with human participants or animals performed by any of authors.

Ethical Approval and Informed Consent Not applicable

References

1. Bridges RA, Berendes H, Good RA (1959) A fatal granulomatous disease of childhood: the clinical, pathological, and laboratory features of a new syndrome. JAMA Pediatr 97(4):387–408
2. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, Franco JL et al (2020) Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol (ePub)
3. Winkelstein JA, Marino MC, Johnston RB Jr, Boyle J, Curnutte J, Gallin JI, Malech HL, Holland SM, Ochs H, Quie P, Buckley RH, Foster CB, Chanock SJ, Dickler H (2000) Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine (Baltimore) 79(3):155–169
4. Rider NL, Jameson MB, Creech CB (2018) Chronic granulomatous disease: epidemiology, pathophysiology, and genetic basis of disease. J pediatric infect dis Soc 7 (suppl_1):S2-S5
5. Wolach B, Gavrieli R, de Boer M, van Leeuwen K, Berger-Achituv S, Stauber T, Ben Ari J, Rottem M, Schlesinger Y, Grisaru-Soen G, Abuzaitoun O, Marcus N, Zion Garty B, Broides A, Levy J, Stepansky P, Etzioni A, Somech R, Roos D (2017) Chronic granulomatous disease: clinical, functional, molecular, and genetic studies. The Israeli experience with 84 patients. Am J Hematol 92(1):28–36
6. Thomas DC, Charbonnier LM, Schejtman A, Aldhekri H, Coomber EL, Dufficy ER, Beenken AE, Lee JC, Clare S, Speak AO, Thrasher AJ, Santilli G, al-Mousa H, Alkuraya FS, Chatila TA, Smith KGC (2019) EROS/CYBC1 mutations: decreased NADPH oxidase function and chronic granulomatous disease. J Allergy Clin Immunol 143(2):782–785 **e781**
7. Thomas DC, Clare S, Sowerby JM, Pardo M, Juss JK, Goulding DA, van der Weyden L, Storisteanu D, Prakash A, Espéli M, Flint S, Lee JC, Hoenderdos K, Kane L, Harcourt K, Mukhopadhyay S, Umrania Y, Antrobus R, Nathan JA, Adams DJ, Bateman A, Choudhary JS, Lyons PA, Condliffe AM, Chilvers ER, Dougan G, Smith KGC (2017) Eros is a novel transmembrane protein that controls the phagocyte respiratory burst and is essential for innate immunity. J Exp Med 214(4):1111–1128
8. Bylund J, Brown KL, Movitz C, Dahlgren C, Karlsson A (2010) Intracellular generation of superoxide by the phagocyte NADPH oxidase: how, where, and what for? Free Radic Biol Med 49(12):1834–1845
9. Dahlgren C, Karlsson A, Bylund J (2019) Intracellular neutrophil oxidants: from laboratory curiosity to clinical reality. J Immunol 202(11):3127–3134
10. Bianchi M, Hakkim A, Brinkmann V, Siler U, Seger RA, Zychlinsky A, Reichenbach J (2009) Restoration of NET formation by gene therapy in CGD controls aspergillosis. Blood 114(13):2619–2622
11. Sharma A, McCann K, Tripathi JK, Chauhan P, Zerbe C, Mishra BB, Holland SM et al (2019) Tamoxifen restores extracellular trap formation in neutrophils from patients with chronic granulomatous disease in a reactive oxygen species-independent manner. J allergy Clin Immunol 144 (2):597-600.e593
12. Trevelin SC, Shah AM, Lombardi G (2020) Beyond bacterial killing: NADPH oxidase 2 is an immunomodulator. Immunol Lett 221:39–48
13. van de Veerdonk FL, Smeekens SP, Joosten LA, Kullberg BJ, Dinarello CA, van der Meer JW, Netea MG (2010) Reactive oxygen species-independent activation of the IL-1beta inflammasome in cells from patients with chronic granulomatous disease. Proc Natl Acad Sci U S A 107(7):3030–3033
14. Meissner F, Seger RA, Moshous D, Fischer A, Reichenbach J, Zychlinsky A (2010) Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease. Blood 116(9):1570–1573
15. Thomas DC (2018) How the phagocyte NADPH oxidase regulates innate immunity. Free Radic Biol Med 125:44–52
16. Deffert C, Carnesecchi S, Yuan H, Rougemont AL, Kelkka T, Holmdahl R, Krause KH, Schäppi MG (2012) Hyperinflammation of chronic granulomatous disease is abolished by NOX2 reconstitution in macrophages and dendritic cells. J Pathol 228(3):341–350

17. Segal BH, Leto TL, Gallin JI, Malech HL, Holland SM (2000) Genetic, biochemical, and clinical features of chronic granulomatous disease. Medicine (Baltimore) 79(3):170–200
18. Kuhns DB, Hsu AP, Sun D, Lau K, Fink D, Griffith P, Huang DW, Priel DAL, Mendez L, Kreuzburg S, Zerbe CS, de Ravin SS, Malech HL, Holland SM, Wu X, Gallin JI (2019) NCF1 (p47(phox))-deficient chronic granulomatous disease: comprehensive genetic and flow cytometric analysis. Blood Adv 3(2):136–147
19. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, Scheible R, Rusch S, Gasteiger LM, Grimbacher B, Mahlaoui N, Ehl S, Abinun M, Albert M, Cohen SB, Bustamante J, Cant A, Casanova JL, Chapel H, de Saint Basile G, de Vries E, Dokal I, Donadieu J, Durandy A, Edgar D, Espanol T, Etzioni A, Fischer A, Gaspar B, Gatti R, Gennery A, Grigoriadou S, Holland S, Janka G, Kanariou M, Klein C, Lachmann H, Lilic D, Manson A, Martinez N, Meyts I, Moes N, Moshous D, Neven B, Ochs H, Picard C, Renner E, Rieux-Laucat F, Seger R, Soresina A, Stoppa-Lyonnet D, Thon V, Thrasher A, van de Veerdonk F, Villa A, Weemaes C, Warnatz K, Wolska B, Zhang SY (2019) The European Society for Immunodeficiencies (ESID) registry working definitions for the clinical diagnosis of inborn errors of immunity. J Allergy Clin Immunol Pract 7(6):1763–1770
20. Ochs HD, Igo RP (1973) The NBT slide test: a simple screening method for detecting chronic granulomatous disease and female carriers. J Pediatr 83(1):77–82
21. Yu JE, Azar AE, Chong HJ, Jongco AM, 3rd, Prince BT (2018) Considerations in the diagnosis of chronic granulomatous disease. J pediatric infect dis Soc 7 (suppl_1):S6-S11
22. Roos D, de Boer M (2014) Molecular diagnosis of chronic granulomatous disease. Clin Exp Immunol 175(2):139–149
23. Kuhns DB, Alvord WG, Heller T, Feld JJ, Pike KM, Marciano BE, Uzel G, DeRavin SS, Priel DAL, Soule BP, Zarember KA, Malech HL, Holland SM, Gallin JI (2010) Residual NADPH oxidase and survival in chronic granulomatous disease. N Engl J Med 363(27):2600–2610
24. van de Geer A, Nieto-Patlan A, Kuhns DB, Tool AT, Arias AA, Bouaziz M, de Boer M et al (2018) Inherited p40phox deficiency differs from classic chronic granulomatous disease. J Clin Invest 128(9):3957–3975
25. Peng J, Redman CM, Wu X, Song X, Walker RH, Westhoff CM, Lee S (2007) Insights into extensive deletions around the XK locus associated with McLeod phenotype and characterization of two novel cases. Gene 392(1–2):142–150
26. Bustamante J, Arias AA, Vogt G, Picard C, Galicia LB, Prando C, Grant AV, Marchal CC, Hubeau M, Chapgier A, de Beaucoudrey L, Puel A, Feinberg J, Valinetz E, Jannière L, Besse C, Boland A, Brisseau JM, Blanche S, Lortholary O, Fieschi C, Emile JF, Boisson-Dupuis S, al-Muhsen S, Woda B, Newburger PE, Condino-Neto A, Dinauer MC, Abel L, Casanova JL (2011) Germline CYBB mutations that selectively affect macrophages in kindreds with X-linked predisposition to tuberculous mycobacterial disease. Nat Immunol 12(3):213–221
27. Ambruso DR, Knall C, Abell AN, Panepinto J, Kurkchubasche A, Thurman G, Gonzalez-Aller C, Hiester A, deBoer M, Harbeck RJ, Oyer R, Johnson GL, Roos D (2000) Human neutrophil immunodeficiency syndrome is associated with an inhibitory Rac2 mutation. Proc Natl Acad Sci 97(9):4654–4659
28. Milligan KL, Mann D, Rump A, Anderson VL, Hsu AP, Kuhns DB, Zerbe CS, Holland SM (2016) Complete myeloperoxidase deficiency: beware the "false-positive" Dihydrorhodamine oxidation. J Pediatr 176:204–206
29. van Bruggen R, Bautista JM, Petropoulou T, de Boer M, van Zwieten R, Gomez-Gallego F, Belohradsky BH et al (2002) Deletion of leucine 61 in glucose-6-phosphate dehydrogenase leads to chronic nonspherocytic anemia, granulocyte dysfunction, and increased susceptibility to infections. Blood 100(3):1026–1030
30. Marciano BE, Spalding C, Fitzgerald A, Mann D, Brown T, Osgood S, Yockey L, Darnell DN, Barnhart L, Daub J, Boris L, Rump AP, Anderson VL, Haney C, Kuhns DB, Rosenzweig SD, Kelly C, Zelazny A, Mason T, DeRavin SS, Kang E, Gallin JI, Malech HL, Olivier KN, Uzel G, Freeman AF, Heller T, Zerbe CS, Holland SM (2015) Common severe infections in chronic granulomatous disease. Clin Infect Dis 60(8):1176–1183
31. Thomsen IP, Smith MA, Holland SM, Creech CB (2016) A comprehensive approach to the Management of Children and Adults with chronic granulomatous disease. J Allergy Clin Immunol Pract 4(6):1082–1088
32. Blancas-Galicia L, Santos-Chavez E, Deswarte C, Mignac Q, Medina-Vera I, Leon-Lara X, Roynard M et al (2020) Genetic, immunological, and clinical features of the first Mexican cohort of patients with chronic granulomatous disease. J Clin Immunol (ePub) 40:475–493
33. Falcone EL, Petts JR, Fasano MB, Ford B, Nauseef WM, Neves JF, Simoes MJ et al (2016) Methylotroph infections and chronic granulomatous disease. Emerg Infect Dis 22(3):404–409
34. Reichenbach J, Lopatin U, Mahlaoui N, Beovic B, Siler U, Zbinden R, Seger RA, Galmiche L, Brousse N, Kayal S, Güngör T, Blanche S, Holland SM (2009) Actinomyces in chronic granulomatous disease: an emerging and unanticipated pathogen. Clin Infect Dis 49(11):1703–1710
35. Leiding JW, Holland SM (2012 [updated 2016]) chronic granulomatous disease. In: Adam MP, Ardinger HH, Pagon RA et al. (eds) GeneReviews® [internet]. Seattle (WA). University of Washington, Seattle; 1993-2020
36. Conti F, Lugo-Reyes SO, Blancas Galicia L, He J, Aksu G, Borges de Oliveira E, Jr., Deswarte C et al (2016) Mycobacterial disease in patients with chronic granulomatous disease: A retrospective analysis of 71 cases. J Allergy Clin Immunol 138 (1): 241–248.e243
37. Haidar G, Zerbe CS, Cheng M, Zelazny AM, Holland SM, Sheridan KR (2017) Phellinus species: an emerging cause of refractory fungal infections in patients with X-linked chronic granulomatous disease. Mycoses 60(3):155–160
38. Sanmun D, Witasp E, Jitkaew S, Tyurina YY, Kagan VE, Ahlin A, Palmblad J et al (2009) Involvement of a functional NADPH oxidase in neutrophils and macrophages during programmed cell clearance: implications for chronic granulomatous disease. Am J Physiol Cell Physiol 297(3):C621–C631
39. Marks DJ, Miyagi K, Rahman FZ, Novelli M, Bloom SL, Segal AW (2009) Inflammatory bowel disease in CGD reproduces the clinicopathological features of Crohn's disease. Am J Gastroenterol 104(1):117–124
40. Connelly JA, Marsh R, Parikh S, Talano JA (2018) Allogeneic hematopoietic cell transplantation for chronic granulomatous disease: controversies and state of the art. J pediatric infect dis Soc 7 (suppl_1):S31-S39
41. Dunogue B, Pilmis B, Mahlaoui N, Elie C, Coignard-Biehler H, Amazzough K, Noel N et al (2017) Chronic granulomatous disease in patients reaching adulthood: a Nationwide study in France. Clin Infect Dis 64(6):767–775

42. Parekh C, Hofstra T, Church JA, Coates TD (2011) Hemophagocytic lymphohistiocytosis in children with chronic granulomatous disease. Pediatr Blood Cancer 56(3):460–462
43. Alvarez-Cardona A, Rodriguez-Lozano AL, Blancas-Galicia L, Rivas-Larrauri FE, Yamazaki-Nakashimada MA (2012) Intravenous immunoglobulin treatment for macrophage activation syndrome complicating chronic granulomatous disease. J Clin Immunol 32(2):207–211
44. Wei A, Ma H, Zhang L, Li Z, Zhang Q, Wang D, Zhang L, Lian H, Zhang R, Wang T (2020) Hemophagocytic lymphohistiocytosis resulting from a cytokine storm triggered by septicemia in a child with chronic granuloma disease: a case report and literature review. BMC Pediatr 20(1):100
45. Bielorai B, Toren A, Wolach B, Mandel M, Golan H, Neumann Y, Kaplinisky C, Weintraub M, Keller N, Amariglio N, Paswell J, Rechavi G (2000) Successful treatment of invasive aspergillosis in chronic granulomatous disease by granulocyte transfusions followed by peripheral blood stem cell transplantation. Bone Marrow Transplant 26(9):1025–1028
46. Marciano BE, Allen ES, Conry-Cantilena C, Kristosturyan E, Klein HG, Fleisher TA, Holland SM, Malech HL, Rosenzweig SD (2017) Granulocyte transfusions in patients with chronic granulomatous disease and refractory infections: the NIH experience. J Allergy Clin Immunol 140(2):622–625
47. Slack MA, Thomsen IP (2018) Prevention of infectious complications in patients with chronic granulomatous disease. J pediatric infect dis Soc 7 (suppl_1):S25-S30
48. Gallin JI, Buescher ES, Seligmann BE, Nath J, Gaither T, Katz P (1983) NIH conference. Recent advances in chronic granulomatous disease. Ann Intern Med 99(5):657–674
49. Margolis DM, Melnick DA, Alling DW, Gallin JI (1990) Trimethoprim-sulfamethoxazole prophylaxis in the management of chronic granulomatous disease. J Infect Dis 162(3):723–726
50. Gallin JI, Alling DW, Malech HL, Wesley R, Koziol D, Marciano B, Eisenstein EM, Turner ML, DeCarlo ES, Starling JM, Holland SM (2003) Itraconazole to prevent fungal infections in chronic granulomatous disease. N Engl J Med 348(24):2416–2422
51. Blumental S, Mouy R, Mahlaoui N, Bougnoux ME, Debre M, Beaute J, Lortholary O et al (2011) Invasive mold infections in chronic granulomatous disease: a 25-year retrospective survey. Clin Infect Dis 53(12):e159–e169
52. A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease. The International Chronic Granulomatous Disease Cooperative Study Group (1991). N Engl J Med 324 (8):509–516
53. Martire B, Rondelli R, Soresina A, Pignata C, Broccoletti T, Finocchi A, Rossi P, Gattorno M, Rabusin M, Azzari C, Dellepiane RM, Pietrogrande MC, Trizzino A, di Bartolomeo P, Martino S, Carpino L, Cossu F, Locatelli F, Maccario R, Pierani P, Putti MC, Stabile A, Notarangelo LD, Ugazio AG, Plebani A, de Mattia D, IPINET. (2008) Clinical features, long-term follow-up and outcome of a large cohort of patients with chronic granulomatous disease: an Italian multicenter study. Clin Immunol 126(2):155–164
54. Naderi beni F, Fattahi F, Mirshafiey A, Ansari M, Mohsenzadegan M, Movahedi M, Pourpak Z et al (2012) Increased production of nitric oxide by neutrophils from patients with chronic granulomatous disease on interferon-gamma treatment. Int Immunopharmacol 12 (4):689–693
55. Oikonomou V, Moretti S, Renga G, Galosi C, Borghi M, Pariano M, Puccetti M, Palmerini CA, Amico L, Carotti A, Prezioso L, Spolzino A, Finocchi A, Rossi P, Velardi A, Aversa F, Napolioni V, Romani L (2016) Noncanonical fungal autophagy inhibits inflammation in response to IFN-gamma via DAPK1. Cell Host Microbe 20(6):744–757
56. Chen LE, Minkes RK, Shackelford PG, Strasberg SM, Kuo EY, Langer JC (2003) Cut it out: managing hepatic abscesses in patients with chronic granulomatous disease. J Pediatr Surg 38(5): 709–713
57. Straughan DM, McLoughlin KC, Mullinax JE, Marciano BE, Freeman AF, Anderson VL, Uzel G et al (2018) The changing paradigm of Management of Liver Abscesses in chronic granulomatous disease. Clin Infect Dis 66(9):1427–1434
58. Uzel G, Orange JS, Poliak N, Marciano BE, Heller T, Holland SM (2010) Complications of tumor necrosis factor-alpha blockade in chronic granulomatous disease-related colitis. Clin Infect Dis 51(12):1429–1434
59. de Luca A, Smeekens SP, Casagrande A, Iannitti R, Conway KL, Gresnigt MS, Begun J, Plantinga TS, Joosten LAB, van der Meer JWM, Chamilos G, Netea MG, Xavier RJ, Dinarello CA, Romani L, van de Veerdonk FL (2014) IL-1 receptor blockade restores autophagy and reduces inflammation in chronic granulomatous disease in mice and in humans. Proc Natl Acad Sci U S A 111(9):3526–3531
60. Hahn KJ, Ho N, Yockey L, Kreuzberg S, Daub J, Rump A, Marciano BE, Quezado M, Malech HL, Holland SM, Heller T, Zerbe CS (2015) Treatment with Anakinra, a recombinant IL-1 receptor antagonist, unlikely to induce lasting remission in patients with CGD colitis. Am J Gastroenterol 110(6):938–939
61. Gabrion A, Hmitou I, Moshous D, Neven B, Lefevre-Utile A, Diana JS, Suarez F et al (2017) Mammalian target of rapamycin inhibition counterbalances the inflammatory status of immune cells in patients with chronic granulomatous disease. J allergy Clin Immunol 139 (5):1641-1649.e1646
62. Fernandez-Boyanapalli RF, Frasch SC, Thomas SM, Malcolm KC, Nicks M, Harbeck RJ, Jakubzick CV et al (2015) Pioglitazone restores phagocyte mitochondrial oxidants and bactericidal capacity in chronic granulomatous disease. J allergy Clin Immunol 135 (2):517-527.e512
63. Hule GP, Bargir UA, Kulkarni M, Kambli P, Taur P, Desai M, Madkaikar MR (2019) Does pioglitazone Lead to neutrophil extracellular traps formation in chronic granulomatous disease patients? Front Immunol 10:1739
64. Fernandez-Boyanapalli R, Frasch SC, Riches DW, Vandivier RW, Henson PM, Bratton DL (2010) PPARgamma activation normalizes resolution of acute sterile inflammation in murine chronic granulomatous disease. Blood 116(22):4512–4522
65. Migliavacca M, Assanelli A, Ferrua F, Cicalese MP, Biffi A, Frittoli M, Silvani P et al (2016) Pioglitazone as a novel therapeutic approach in chronic granulomatous disease. J allergy Clin Immunol 137 (6):1913-1915.e1912
66. Douda DN, Khan MA, Grasemann H, Palaniyar N (2015) SK3 channel and mitochondrial ROS mediate NADPH oxidase-independent NETosis induced by calcium influx. Proc Natl Acad Sci U S A 112(9):2817–2822
67. Ahlin A, Fasth A (2015) Chronic granulomatous disease - conventional treatment vs. hematopoietic stem cell transplantation: an update. Curr Opin Hematol 22(1):41–45
68. Cole T, McKendrick F, Titman P, Cant AJ, Pearce MS, Cale CM, Goldblatt D, Gennery AR (2013) Health related quality of life and emotional health in children with chronic granulomatous disease: a comparison of those managed conservatively with those that have undergone haematopoietic stem cell transplant. J Clin Immunol 33(1):8–13

69. Gragert L, Eapen M, Williams E, Freeman J, Spellman S, Baitty R, Hartzman R, Rizzo JD, Horowitz M, Confer D, Maiers M (2014) HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. N Engl J Med 371(4):339–348
70. de la Morena MT, Nelson RP Jr (2014) Recent advances in transplantation for primary immune deficiency diseases: a comprehensive review. Clin Rev Allergy Immunol 46(2):131–144
71. Kekre N, Antin JH (2014) Hematopoietic stem cell transplantation donor sources in the 21st century: choosing the ideal donor when a perfect match does not exist. Blood 124(3):334–343
72. Shouval R, Fein JA, Labopin M, Kröger N, Duarte RF, Bader P, Chabannon C, Kuball J, Basak GW, Dufour C, Galimard JE, Polge E, Lankester A, Montoto S, Snowden JA, Styczynski J, Yakoub-Agha I, Mohty M, Nagler A (2019) Outcomes of allogeneic haematopoietic stem cell transplantation from HLA-matched and alternative donors: a European Society for Blood and Marrow Transplantation registry retrospective analysis. The Lancet Haematology 6(11):e573–e584
73. Klein OR, Chen AR, Gamper C, Loeb D, Zambidis E, Llosa N, Huo J, Dezern AE, Steppan D, Robey N, Holuba MJ, Cooke KR, Symons HJ (2016) Alternative-donor hematopoietic stem cell transplantation with post-transplantation cyclophosphamide for nonmalignant disorders. Biol Blood Marrow Transplant 22(5): 895–901
74. Hoenig M, Niehues T, Siepermann K, Jacobsen EM, Schutz C, Furlan I, Duckers G et al (2014) Successful HLA haploidentical hematopoietic SCT in chronic granulomatous disease. Bone Marrow Transplant 49(10):1337–1338
75. Morillo-Gutierrez B, Beier R, Rao K, Burroughs L, Schulz A, Ewins AM, Gibson B, Sedlacek P, Krol L, Strahm B, Zaidman I, Kalwak K, Talano JA, Woolfrey A, Fraser C, Meyts I, Müller I, Wachowiak J, Bernardo ME, Veys P, Sykora KW, Gennery AR, Slatter M (2016) Treosulfan-based conditioning for allogeneic HSCT in children with chronic granulomatous disease: a multicenter experience. Blood 128(3):440–448
76. Arnold DE, Seif AE, Jyonouchi S, Sullivan KE, Bunin NJ, Heimall JR (2019) Allogeneic hematopoietic stem cell transplantation in adolescent patients with chronic granulomatous disease. J Allergy Clin Immunol Pract 7(3):1052–1054 **e1052**
77. Yonkof JR, Gupta A, Fu P, Garabedian E, Dalal J (2019) Role of allogeneic hematopoietic stem cell transplant for chronic granulomatous disease (CGD): a report of the United States Immunodeficiency Network. J Clin Immunol 39(4):448–458
78. Gungor T, Teira P, Slatter M, Stussi G, Stepensky P, Moshous D, Vermont C et al (2014) Reduced-intensity conditioning and HLA-matched haemopoietic stem-cell transplantation in patients with chronic granulomatous disease: a prospective multicentre study. Lancet 383(9915):436–448
79. Marciano BE, Zerbe CS, Falcone EL, Ding L, DeRavin SS, Daub J, Kreuzburg S et al (2018) X-linked carriers of chronic granulomatous disease: illness, lyonization, and stability. J Allergy Clin Immunol 141(1):365–371
80. Battersby AC, Braggins H, Pearce MS, Cale CM, Burns SO, Hackett S, Hughes S et al (2017) Inflammatory and autoimmune manifestations in X-linked carriers of chronic granulomatous disease in the United Kingdom. J allergy Clin Immunol 140 (2):628-630.e626
81. Marsh RA, Leiding JW, Logan BR, Griffith LM, Arnold DE, Haddad E, Falcone EL et al (2019) Chronic granulomatous disease-associated IBD resolves and does not adversely impact survival following allogeneic HCT. J Clin Immunol 39(7):653–667
82. Ott MG, Schmidt M, Schwarzwaelder K, Stein S, Siler U, Koehl U, Glimm H, Kühlcke K, Schilz A, Kunkel H, Naundorf S, Brinkmann A, Deichmann A, Fischer M, Ball C, Pilz I, Dunbar C, du Y, Jenkins NA, Copeland NG, Lüthi U, Hassan M, Thrasher AJ, Hoelzer D, von Kalle C, Seger R, Grez M (2006) Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. Nat Med 12(4):401–409
83. Kang EM, Choi U, Theobald N, Linton G, Long Priel DA, Kuhns D, Malech HL (2010) Retrovirus gene therapy for X-linked chronic granulomatous disease can achieve stable long-term correction of oxidase activity in peripheral blood neutrophils. Blood 115(4): 783–791
84. Kohn DB, Booth C, Kang EM, Pai SY, Shaw KL, Santilli G, Armant M et al (2020) Lentiviral gene therapy for X-linked chronic granulomatous disease. Nat med (ePub)
85. Joung JK, Sander JD (2013) TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol 14(1): 49–55
86. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD (2010) Genome editing with engineered zinc finger nucleases. Nat Rev Genet 11(9):636–646
87. Doudna JA, Charpentier E (2014) Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346 (6213):1258096
88. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR (2016) Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 533(7603):420–424
89. Klatt D, Cheng E, Philipp F, Selich A, Dahlke J, Schmidt RE, Schott JW, Büning H, Hoffmann D, Thrasher AJ, Schambach A (2019) Targeted repair of p47-CGD in iPSCs by CRISPR/Cas9: functional correction without cleavage in the highly homologous Pseudogenes. Stem Cell Reports 13(4):590–598
90. Battersby AC, Cale AM, Goldblatt D, Gennery AR (2013) Clinical manifestations of disease in X-linked carriers of chronic granulomatous disease. J Clin Immunol 33(8):1276–1284
91. Cale CM, Morton L, Goldblatt D (2007) Cutaneous and other lupus-like symptoms in carriers of X-linked chronic granulomatous disease: incidence and autoimmune serology. Clin Exp Immunol 148(1):79–84
92. Hauck F, Koletzko S, Walz C, von Bernuth H, Klenk A, Schmid I, Belohradsky BH et al (2016) Diagnostic and treatment options for severe IBD in female X-CGD carriers with non-random X-inactivation. J Crohns Colitis 10(1):112–115
93. Ahlin A, Fugelang J, de Boer M, Ringden O, Fasth A, Winiarski J (2013) Chronic granulomatous disease-haematopoietic stem cell transplantation versus conventional treatment. Acta Paediatr 102(11):1087–1094
94. Khandelwal P, Bleesing JJ, Davies SM, Marsh RA (2016) A single-center experience comparing Alemtuzumab, Fludarabine, and Melphalan reduced-intensity conditioning with Myeloablative Busulfan, cyclophosphamide, and antithymocyte globulin for chronic granulomatous disease. Biol Blood Marrow Transplant 22(11):2011–2018
95. Zou J, Sweeney CL, Chou BK, Choi U, Pan J, Wang H, Dowey SN, Cheng L, Malech HL (2011) Oxidase-deficient neutrophils from X-linked chronic granulomatous disease iPS cells: functional correction by zinc finger nuclease-mediated safe harbor targeting. Blood 117(21):5561–5572
96. Flynn R, Grundmann A, Renz P, Hanseler W, James WS, Cowley SA, Moore MD (2015) CRISPR-mediated genotypic and

phenotypic correction of a chronic granulomatous disease mutation in human iPS cells. Exp Hematol 43 (10):838-848.e833

97. Merling RK, Sweeney CL, Chu J, Bodansky A, Choi U, Priel DL, Kuhns DB, Wang H, Vasilevsky S, de Ravin SS, Winkler T, Dunbar CE, Zou J, Zarember KA, Gallin JI, Holland SM, Malech HL (2015) An AAVS1-targeted minigene platform for correction of iPSCs from all five types of chronic granulomatous disease. Mol Ther 23(1):147–157
98. Dreyer AK, Hoffmann D, Lachmann N, Ackermann M, Steinemann D, Timm B, Siler U, Reichenbach J, Grez M, Moritz T, Schambach A, Cathomen T (2015) TALEN-mediated functional correction of X-linked chronic granulomatous disease in patient-derived induced pluripotent stem cells. Biomaterials 69: 191–200
99. De Ravin SS, Reik A, Liu PQ, Li L, Wu X, Su L, Raley C et al (2016) Targeted gene addition in human CD34(+) hematopoietic cells for correction of X-linked chronic granulomatous disease. Nat Biotechnol 34(4):424–429
100. De Ravin SS, Li L, Wu X, Choi U, Allen C, Koontz S, Lee J et al (2017) CRISPR-Cas9 gene repair of hematopoietic stem cells from patients with X-linked chronic granulomatous disease. Sci Transl Med 9(372):eaah3480
101. Sweeney CL, Zou J, Choi U, Merling RK, Liu A, Bodansky A, Burkett S, Kim JW, de Ravin SS, Malech HL (2017) Targeted repair of CYBB in X-CGD iPSCs requires retention of Intronic sequences for expression and functional correction. Mol Ther 25(2):321–330