on these results, CTLA-4 haploinsufficiency was considered. *CTLA4* sequencing revealed a previously published pathogenic variant, c.223C>T (p.R75W).[2] Sequencing revealed the same mutation in her asymptomatic mother.

At the age of 13, the patient underwent an excisional biopsy of a presumed submandibular lymph node at another institution. The histopathology revealed a submandibular salivary gland with destruction of nearly all of the acini, preservation of residual salivary gland ducts, thick bands of collagen fibrosis, and abundant ectopic (inducible) mucosa-associated lymphoid tissue (MALT) (Fig 1). The ectopic MALT contained expanded secondary lymphoid follicles with fully developed architecture, which contained germinal centers with a mixture of follicular center cells, follicular dendritic cells, and PD1-positive follicular helper/regulatory T cells (TFH and TFR cannot be distinguished by routine immunohistochemistry). The borders between mantle zones and germinal centers were distinct, and intermingling of mantle cells and follicular center cells, characteristic of progressive transformation of germinal centers, was not present. There was no obvious increase in DNTs in paracortical zones, and no evidence of MALT lymphoma or follicular lymphoma.

In vitro studies of peripheral blood TFH, TFR, and Treg lymphocytes were performed as published previously[8] and revealed a normal percentage of Tregs with decreased CD25 and CTLA-4 expression in both patient and mother (see Fig E1, *A* in this article's Online Repository at www.jacionline.org). Further studies demonstrated increased TFH cells with concomitantly decreased TFR cells in peripheral blood (see Fig E1, *B*) and defective Treg suppression in the patient, compared with healthy controls. Despite the same *CTLA4* mutation, these studies were normal in the mother (see Fig E1, *C*). Additionally, the patient's circulating TFH cells expressed more IL-21, but not IL-2, than healthy controls did, suggesting a more activated status of TFH cells in the patient (see Fig E1, *B*).

This case highlights the need to screen for CTLA-4 haploinsufficiency in patients with autoimmune lymphoproliferative disorders, resembling ALPS, who not meet ALPS criteria. It also emphasizes 2 important features: distinct histopathology and a distinct Treg, TFH, and TFR profile in CTLA-4 haploinsufficiency.

With regard to the former, our finding of ectopic (inducible) MALT in a nonlymphoid organ, with extensive collage fibrosis, as well as an absence of progressive transformation of germinal centers and an absence of an overtly expanded DNT population, raise the possibility that patients with CTLA-4 haploinsufficiency have a histopathology that is distinctive from patients with typical ALPS.[9] Consistent with this conclusion, a lung biopsy from another patient with the same R75W mutation in *CTLA4* revealed similar findings, including "lymphoid fibrotic lesions," ectopic MALT with secondary lymphoid follicles with germinal centers, and a predominance of CD4-positive T cells in between the follicles.[2]

With regard to the latter, our results showing a profile of increased TFH cells with low frequency of TFR cells, linked to impaired Treg suppression, might serve as a biomarker profile for disease (prediction) in individuals with *CTLA4* mutations. This is relevant given the published variable penetrance of disease in families and perhaps indicates the existence of yet unknown modifying genes.[2,4] Lastly, our results invite further analysis of TFH, TFR, and Treg populations in the broader context of classification of ALPS and related diseases.

In conclusion, our detailed clinical, immunologic, and histopathologic, characterization adds to a growing body of data indicating an important role for CTLA-4 in the regulation of the germinal center response. This is likely relevant for immunologic features in these patients, such as autoimmunity and an increased risk of lymphoma, and might facilitate a better demarcation of CTLA-4 haploinsufficiency from other autoimmune lymphoproliferative disorders and, ultimately, a more accurate and pathogenesis-driven classification of these rare disorders.

Zeynep Yesim Kucuk, MD[a]
Louis-Marie Charbonnier, PhD[b]
Richard L. McMasters, MD[c]
Talal Chatila, MD, MSc[b]
Jack J. Bleesing, MD, PhD[a]

From [a]the Division of Bone Marrow Transplantation and Immune Deficiency, and [c]the Division of Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; and [b]the Division of Allergy and Immunology, Boston Children's Hospital, Boston, Mass. E-mail: zeynep.kucuk@cchmc.org.

Supported by the National Institutes of Health (#R01AI085090 to T.C.).
Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Oliveira JB, Bleesing JJ, Dianzani U, Fleisher TA, Jaffe ES, Lenardo MJ, et al. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop. Blood 2010;116:e35-40.
2. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med 2014;20:1410-6.
3. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science 2008;322:271-5.
4. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT, et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. Science 2014;345:1623-7.
5. Chung Y, Tanaka S, Chu F, Nurieva RI, Martinez GJ, Rawal S, et al. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. Nat Med 2011;17:983-8.
6. Sage PT, Paterson AM, Lovitch SB, Sharpe AH. The coinhibitory receptor CTLA-4 controls B cell responses by modulating T follicular helper, T follicular regulatory, and T regulatory cells. Immunity 2014;41:1026-39.
7. Ma CS, Wong N, Rao G, Avery DT, Torpy J, Hambridge T, et al. Monogenic mutations differentially affect the quantity and quality of T follicular helper cells in patients with human primary immunodeficiencies. J Allergy Clin Immunol 2015;136:993-1006.e1.
8. Charbonnier LM, Janssen E, Chou J, Ohsumi TK, Keles S, Hsu JT, et al. Regulatory T-cell deficiency and immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like disorder caused by loss-of-function mutations in LRBA. J Allergy Clin Immunol 2015;135:217-27.
9. Lim MS, Straus SE, Dale JK, Fleisher TA, Stetler-Stevenson M, Strober W, et al. Pathological findings in human autoimmune lymphoproliferative syndrome. Am J Pathol 1998;153:1541-50.

Available online March 30, 2017.
http://dx.doi.org/10.1016/j.jaci.2017.02.032

Omalizumab is effective in cold urticaria—results of a randomized placebo-controlled trial

To the Editor:

Cold urticaria, a common and dangerous form of chronic inducible urticaria, is characterized by itchy wheals, angioedema, or both that typically occur within minutes at cold-exposed skin and mucosal sites.[1] Cold urticaria accounts for one-third of all cases of physical urticaria, and it is the most dangerous urticaria disease with potential loss of consciousness and death due to extensive cold contact.[2] Up to 72% of patients with cold urticaria

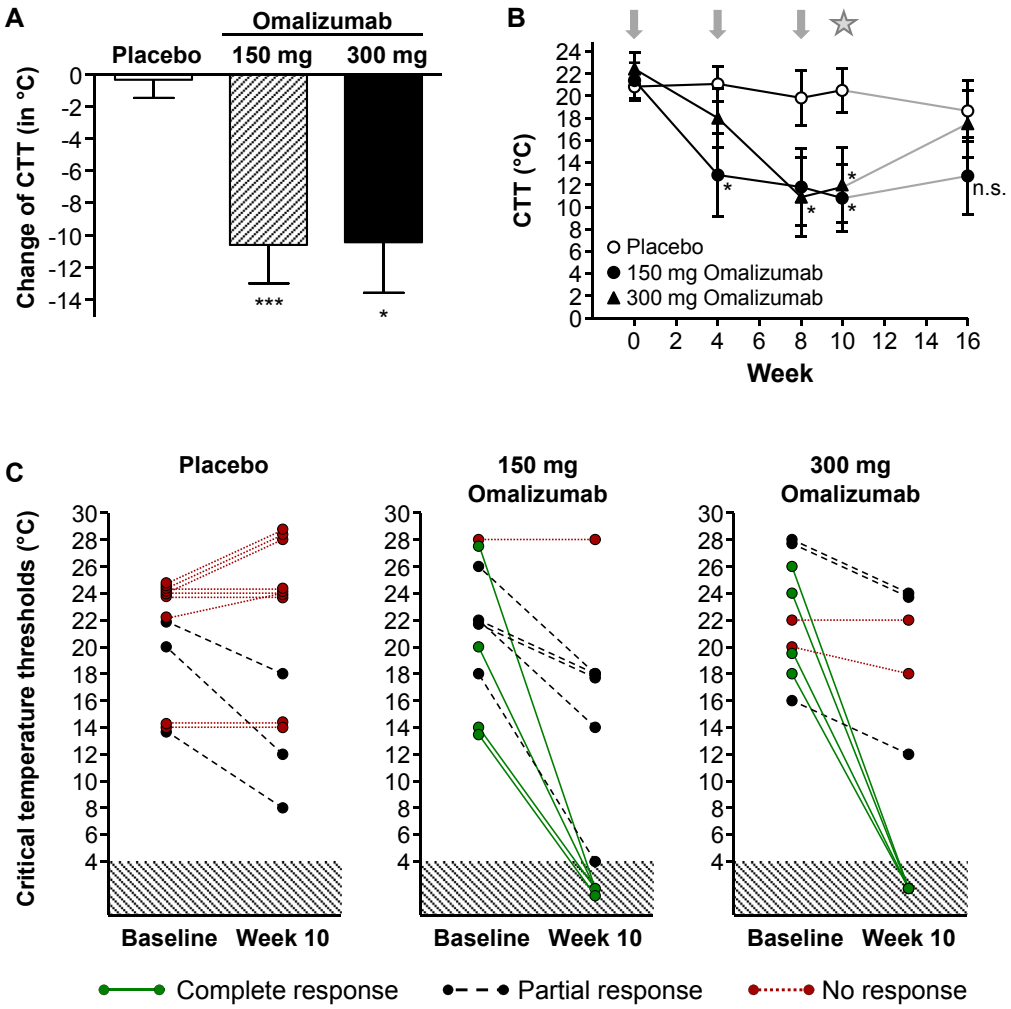

FIG 1. Omalizumab is effective in cold urticaria. CTTs were assessed using TempTest and mean reduction in CTTs from baseline at the primary end point at week 10 **(A)** and mean absolute values at every investigated time point are shown **(B)**. Omalizumab 150 mg, omalizumab 300 mg, or placebo was injected subcutaneously 3 times every 4 weeks (indicated by an *arrow*). The *star* indicates the primary end point readout 2 weeks after the last injection. **C,** Individual provocation thresholds before and after treatment. Individual CTTs before and after treatment were assessed using TempTest. *n.s.*, Not significant. $*P < .05$, $**P < .01$, and $***P < .005$.

experience systemic reactions after extensive cold contact.[2] The underlying cause of cold urticaria is as of yet largely unknown. However, at least in some patients, IgE has been shown to be a relevant factor.[3]

Because the trigger of cold urticaria, that is, cold exposure, is difficult, if not impossible, to avoid in everyday life, effective symptomatic treatment is needed. In many patients, antihistamine treatment, even with higher than standard doses, is not sufficient to control the occurrence of cold urticaria signs and symptoms.[4,5] Several case reports suggest that patients with cold urticaria can benefit from treatment with omalizumab, a humanized anti-IgE antibody.[6,7] Here, we report the results of an investigator-initiated multicenter phase 2 trial evaluating the efficacy and safety of 2 different doses of omalizumab as compared with placebo in patients with cold urticaria refractory to antihistamine treatment.

Thirty-one patients with cold urticaria were recruited at 3 study centers, and all patients were included in the primary end point and safety analysis (for details on study procedures, see this article's Online Repository and Fig E1 at www.jacionline.org). The planned number of patients to be included was 60, but an independent blinded interim analysis was performed for ethical reasons after half of all planned patients had been included. Cold urticaria is a potentially life-threatening disease and placebo treatment of patients does not provide protection from cold-induced systemic reactions including anaphylactic shock. Because the interim analysis showed marked clinical and statistical superiority of omalizumab compared with placebo, the study was terminated. The baseline, demographic, and clinical characteristics were comparable in all groups (see Table E1 in this article's Online Repository at www.jacionline.org).

Mean changes from baseline in the individual temperatures required to induce symptoms (ie, critical temperature thresholds [CTTs]) at week 10 (primary end point) were markedly and significantly higher (Fig 1, *A*) in the groups receiving 150 mg omalizumab ($-10.6°C \pm 2.4°C$; $P = .001$) and 300 mg omalizumab ($-10.4°C \pm 3.1°C$; $P = .013$), compared with placebo ($-0.3°C \pm 1.1°C$) (Fig 1, *A*). The treatment with omalizumab

resulted in a rapid reduction in CTTs, with first improvements seen as early as 4 weeks after the first treatment (Fig 1, *B*). In the follow-up period after the last injection, CTTs in the omalizumab groups increased and did not show differences to those in the placebo group 6 weeks after the last injections. No significant difference between 150 mg and 300 mg omalizumab was observed at any time during the study (Fig 1, *A* and *B*).

Individual CTTs can differ considerably. Therefore, we additionally assessed the CTTs in every patient at baseline and 10 weeks later (Fig 1, *C*). Patients with low and high baseline CTTs showed similar responses to omalizumab treatment. Furthermore, the rate of patients responding to treatment is an important parameter of treatment efficacy. The responder analysis showed significant differences between the groups ($P = .012$, χ^2 test). The rate of patients with cold urticaria with complete response at week 10 after placebo treatment was 0 of 12 (0%), whereas 4 of 10 (40%) showed complete response to 150 mg omalizumab and 4 of 9 (44%) to 300 mg omalizumab (Fig 1, *C*). No response at all was observed in 9 of 12 (75%) patients treated with placebo, 1 of 10 (10%) patients treated with omalizumab 150 mg, and 2 of 9 (22%) patients treated with omalizumab 300 mg (Fig 1, *C*). The frequency of adverse events was similar across all groups, and no serious adverse event occurred in any of the treatment groups (see Table E2 in this article's Online Repository at www.jacionline.org).

Here, we show that omalizumab is effective in cold urticaria with effect sizes that are comparable to those in patients with chronic spontaneous urticaria treated with omalizumab. Furthermore, the rate of complete responders, that is, patients with no symptoms at all, is comparable in clinical trials of omalizumab in chronic spontaneous urticaria (34% to 40%)[8] and our studies in cold urticaria (44%), indicating overall similar efficacy of omalizumab in patients with chronic spontaneous urticaria and cold urticaria refractory to antihistamine treatment.

Interestingly, omalizumab was found to be similarly effective in both doses, 150 mg and 300 mg, with no statistical differences between the 2 treatment groups. This is in contrast to chronic spontaneous urticaria, in which also both doses have been reported to be effective, but 300 mg omalizumab has been shown to be more effective than 150 mg omalizumab in the reduction of symptoms.[8] In chronic spontaneous urticaria, the efficacy of omalizumab appears to be largely independent of the levels of total IgE. This has led to the licensing of a fixed dose (300 mg) for treatment in chronic spontaneous urticaria irrespective of the levels of total IgE. Whether this may be different in cold urticaria, and thus whether nonresponders to omalizumab may have different levels of IgE as responders, we cannot answer here. In a comparison of responders and partial responders or nonresponders (see Table E3 in this article's Online Repository at www.jacionline.org), more atopic patients were seen in the group of complete responders (5 of 8 vs 1 of 11, respectively; $P = .04$). The number of patients in this comparison is, however, too low to draw a final conclusion. Future clinical trials with more patients will have to be performed to answer this and to show whether 150 mg omalizumab is, in contrast to chronic spontaneous urticaria, the optimal dose. Interestingly, the efficacy of omalizumab in cold urticaria was found to be similar irrespective of the CTTs at baseline, indicating that disease severity is not a predictor of treatment response.

It is as of yet unknown why omalizumab has these strong effects on disease activity in cold urticaria. The relevance of IgE is still controversially discussed and the pathophysiologic role of IgE in chronic inducible urticaria in general is largely unknown. Experiments using passive serum transfer have suggested that a soluble transferable factor, presumably IgE, might be involved, in some patients, in the pathogenesis of physical urticaria forms such as symptomatic dermographism, solar urticaria, and cold urticaria.[3,9,10] The marked efficacy of anti-IgE treatment seen here argues in favor of a pathogenetic role for IgE in cold urticaria. This could be a specific IgE, which is directed against a skin-derived protein that becomes accessible on stimulation by cold. Future investigations need to be carried out to better characterize the role of IgE in cold urticaria pathogenesis and the mechanism of action of omalizumab in cold urticaria. Also, additional controlled trials are needed to test whether omalizumab is similarly effective in other chronic inducible urticaria types such as solar, heat, or pressure urticaria, diseases that all have been described in case reports to be effectively treated by omalizumab.

In conclusion, omalizumab in doses of 150 mg and 300 mg resulted in a high rate of complete and partial responders in patients with cold urticaria and a pronounced overall reduction in disease activity. The results of the studies show that patients with cold urticaria unresponsive to antihistamine treatment can benefit from a well-tolerated and effective treatment with omalizumab.

We thank Hesna Gözlükaya and Nikki Rooks for excellent technical assistance and the team of the Charité urticaria clinic, a GA^{2}LEN Urticaria Center of Reference and Excellence (UCARE, www.ga2len.net/ucare), for support and helpful discussions. The trial was an investigator-initiated trial funded, in part, by Novartis and by intramural grants. M. Metz is supported by the Else Kröner-Fresenius-Stiftung.

Martin Metz, MD[a]
Andrea Schütz, MD[a]
Karsten Weller, MD[a]
Marina Gorczyza, MD[a]
Sebastian Zimmer, MD[b]
Petra Staubach, MD[b]
Hans F. Merk, MD[c]
Marcus Maurer, MD[a]

From [a]the Department of Dermatology and Allergy, Charité – Universitätsmedizin Berlin, Berlin, Germany; [b]the Department of Dermatology, University Medical Center Mainz, Mainz, Germany; and [c]the Department of Dermatology and Allergy, RWTH Aachen University, Aachen, Germany. E-mail: marcus.maurer@charite.de.

The trial was an investigator-initiated trial funded, in part, by Novartis and by intramural grants. Novartis had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Disclosure of potential conflict of interest: M. Metz has received grants from Novartis and Else Kröner-Fresenius Foundation; has consultant arrangements with Novartis, Bayer, and Nerre Therapeutics; has received payment for lectures from Moxie, Novartis, and Roche; and has received travel support from Shire. K. Weller has received a grant from, has a board membership with, and has consultant arrangements with Novartis; has received grants from Essex Pharma (now MSD), FAES, and Uriach; has received payment for lectures from Dr. R. Pfleger, Essex Pharma (now MSD), Uriach, UCB, Novartis, and Moxie; and has received travel support from Novartis. P. Staubach has board memberships with Novartis, Genentech, Abbvie, Janssen, Leti, and Sobi; has consultant arrangements with Novartis; has received grants from Novartis; has received payment for lectures from Pfleger, MSD, Leti, Abbvie, Janssen, and Shire; and has received travel support from Novartis and Janssen. M. Maurer has received grants from Novartis, Uriach, FAES, and Moxie; has consultant arrangements with Novartis, Genentech, Uriach, FAES, and Moxie; and has received payment for lectures from Novartis, Genentech, Uriach, and FAES. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Magerl M, Altrichter S, Borzova E, Gimenez-Arnau A, Grattan CE, Lawlor F, et al. The definition, diagnostic testing, and management of chronic inducible urticarias—the EAACI/GA(2) LEN/EDF/UNEV consensus recommendations 2016 update and revision. Allergy 2016;71:780-802.

2. Abajian M, Mlynek A, Maurer M. Physical urticaria. Curr Allergy Asthma Rep 2012;12:281-7.
3. Kaplan AP, Garofalo J, Sigler R, Hauber T. Idiopathic cold urticaria: in vitro demonstration of histamine release upon challenge of skin biopsies. N Engl J Med 1981;305:1074-7.
4. Siebenhaar F, Weller K, Mlynek A, Magerl M, Altrichter S, Vieira Dos Santos R, et al. Acquired cold urticaria: clinical picture and update on diagnosis and treatment. Clin Exp Dermatol 2007;32:241-5.
5. Singleton R, Halverstam CP. Diagnosis and management of cold urticaria. Cutis 2016;97:59-62.
6. Boyce JA. Successful treatment of cold-induced urticaria/anaphylaxis with anti-IgE. J Allergy Clin Immunol 2006;117:1415-8.
7. Metz M, Ohanyan T, Church MK, Maurer M. Omalizumab is an effective and rapidly acting therapy in difficult-to-treat chronic urticaria: a retrospective clinical analysis. J Dermatol Sci 2014;73:57-62.
8. Casale TB, Bernstein JA, Maurer M, Saini SS, Trzaskoma B, Chen H, et al. Similar efficacy with omalizumab in chronic idiopathic/spontaneous urticaria despite different background therapy. J Allergy Clin Immunol Pract 2015;3:743-50.e1.
9. Grabbe J. Pathomechanisms in physical urticaria. J Investig Dermatol Symp Proc 2001;6:135-6.
10. Newcomb RW, Nelson H. Dermographia mediated by immunoglobulin E. Am J Med 1973;54:174-80.

Available online April 4, 2017.
http://dx.doi.org/10.1016/j.jaci.2017.01.043

IL-10 overexpression predisposes to invasive aspergillosis by suppressing antifungal immunity

To the Editor:

Proinflammatory immune responses are critically required for antimicrobial host defenses; however, excessive inflammation has the potential to damage host tissues thereby paradoxically contributing to the progression of infection. A central negative regulator of inflammatory responses is IL-10, an immunosuppressive cytokine with a wide variety of functions across multiple cell types.[1] Although the role of IL-10 during infection appears to vary for different microorganisms, a largely detrimental role has been attributed to this cytokine during fungal disease.[2] Given the variable risk of infection and its outcome among patients with comparable predisposing factors, susceptibility to invasive aspergillosis (IA) is thought to rely largely on genetic predisposition.[3] The initial investigation of genetic variability at the *IL10* locus led to the identification of single nucleotide polymorphisms (SNPs) influencing its transcriptional activity; thus, IL-10 may be a reasonable candidate for the genetic regulation of susceptibility to IA in high-risk patients.

In a 2-stage, multicenter study involving 413 hematopoietic stem-cell transplantation (HSCT) donor-recipient pairs, we confirmed that SNPs in *IL10* are critical regulators of susceptibility to IA. Using a discovery cohort of donor-recipient pairs (see Table E1 in this article's Online Repository at www.jacionline.org), we analyzed 5 haplotype-tagging SNPs in *IL10* (see Table E2 in this article's Online Repository at www.jacionline.org) and found that the donor, but not recipient, rs1800896 SNP was associated with an increased risk of IA (Fig 1, *A* and see Table E3 in this article's Online Repository at www.jacionline.org). The contribution of the GG genotype to the risk of infection was further illustrated on modeling a recessive mode of inheritance (Fig 1, *B*). In a multivariate model, the donor GG genotype conferred a 2.6-fold increased risk of developing IA after transplantation, and the association test results were further validated in a confirmation case-control study involving patients with similar demographic and clinical characteristics and by a meta-analysis including all enrolled patients (see Table E4 in this article's Online Repository at www.jacionline.org). Furthermore, although no significant differences were observed, the probability of infection-free survival in the discovery set decreased from 88% among patients with the AA genotype to 79% and 75% among subjects carrying the AG or GG genotypes, respectively (Fig 1, *C*).

Using sequence data from the 1000 Genomes Project, we identified all SNPs in linkage disequilibrium (LD) with rs1800896 (see Table E5 in this article's Online Repository at www.jacionline.org), but none of these were exonic. LD around this SNP was limited to the *IL10* locus (see Fig E1 in this article's Online Repository at www.jacionline.org), implying that noncoding variation is likely to drive the association with IA. To ascertain whether rs1800896, or a variant in strong LD with it, influenced gene transcription, we monitored IL-10 mRNA and protein expression in PBMCs from healthy blood donors subjected to *in vitro* infection with *Aspergillus fumigatus*. We observed striking genotype-specific differences, with PBMCs carrying the GG genotype expressing higher transcript and protein levels than those from AA or AG carriers (Fig 2, *A*). A similarly enhanced IL-10 production was observed in monocyte-derived macrophages from GG carriers (Fig 2, *B*), and the overexpression phenotype was independent of specific pattern recognition receptor activation (Fig 2, *C*). Although the ability of macrophages to ingest the conidia remained intact regardless of the genotype (Fig 2, *D*), cells carrying the IL-10 high-producing genotype displayed a 25% decrease in their ability to clear the fungus, as compared to AA carriers (Fig 2, *E*). This defect was dependent on IL-10, because inhibiting IL-10-mediated signals with a neutralizing antibody restored the fungicidal ability. Importantly, the donor GG genotype also differentially regulated the levels of IL-10 in hematological patients, with higher levels present in bronchoalveolar lavages from cases of IA carrying the GG genotype than AA carriers (Fig 2, *F*).

In contrast to IL-10, PBMCs carrying the GG genotype at rs1800896 secreted lower amounts of TNF-α than those from AA or AG carriers after infection (Fig 2, *G*), a finding implying that, under these conditions, GG homozygotes generate lesser inflammatory responses. The dichotomy between IL-10 and TNF-α production according to rs1800896 genotypes was confirmed in human macrophages, in which the same genotype-specific alterations were observed (Fig 2, *H*) and extended to other proinflammatory cytokines such as IL-6, IL-1β, and IL-8 (Fig 2, *I*). Strikingly, we observed that the defect in TNF-α production by macrophages from GG carriers was abolished when IL-10 was neutralized (Fig 2, *J*). In support of this, the addition of IL-10 to cells carrying low-producing genotypes restrained the production of TNF-α in response to infection (Fig 2, *K*). Likewise, the median concentrations of TNF-α were also decreased among hematological patients carrying the IL-10 high-producing genotype (Fig 2, *L*).

We have identified rs1800896 (or a variant in strong LD with it) as the underlying causal variant within the *IL10* locus and disclosed the suppression of immune responses to *A fumigatus* as the primary mechanism explaining the increased susceptibility to infection. The rs1800896 alleles have been shown to physically interact with the transcription repressor poly(adenosine diphosphate–ribose) polymerase 1 and the specificity protein 1 in an

METHODS

We performed an investigator-initiated, multicenter, randomized, double-blind, placebo-controlled study to investigate the efficacy and safety of 2 doses of omalizumab over 16 weeks in adult patients with antihistamine-refractory cold urticaria (CUTEX trial). The trial was conducted in university hospitals in Berlin, Mainz, and Aachen, all in Germany. The study protocol was approved by all relevant ethics committees and the German regulatory authority for mAbs (Paul Ehrlich Institut) and was conducted in accordance with the Declaration of Helsinki. The study has been registered with EudraCT (2011-003746-41) and with ClinicalTrials.gov (NCT 01580592). Participants were recruited from July 1, 2012, to December 31, 2014.

Patients

All patients were and had to be between 18 and 75 years old with a history of at least 6 months of cold urticaria refractory to antihistamine treatment. Exclusion criteria included treatment with systemic steroids, cyclosporine, methotrexate, dapsone, or other immunosuppressives within the previous 4 weeks, the use of any antihistamine or leukotriene antagonist 4 days before visit 1, a history of malignancies within 5 years before the screening visit, a known hypersensitivity to omalizumab, previous treatment with omalizumab, and pregnancy. Before inclusion, all patients provided written informed consent.

Randomization and masking

After a 1- to 2-week screening period, patients were randomly assigned to receive omalizumab 150 mg, omalizumab 300 mg, or placebo in a ratio of 1:1:1. No stratification was used. Patients were enrolled by the respective study centers and randomized centrally to the treatment arms using a randomization list provided by the study drug provider (Novartis, Basel, Switzerland), who was not involved in the rest of the trial. Blinding of study medication was achieved by using placebo containing the same ingredients as the omalizumab formulation, excluding omalizumab. There were no visible differences between omalizumab and placebo; however, the viscosity of the 2 differed. Therefore, a separate and independent unblinded study team, which did not engage in study-specific communication with the blinded study team, was responsible for the preparation and injection of the study drug. Unblinded study team members were not involved in any other parts of the trials.

Procedures

Each patient received 3 subcutaneous injections of 150 mg omalizumab, 300 mg omalizumab, or placebo in 4-week intervals, with the primary readout 2 weeks after the last injection. This 10-week treatment period was followed by a 6-week follow-up period. At the end of week 0, 4, 8, 10, and 16, patients underwent provocation tests at the study center to assess trigger thresholds. For the determination of CTTs, TempTest 4.0 (Courage + Khazaka Electronics GmbH, Cologne, Germany) was used, a validated device for standardized and reproducible cold provocation.[E1]

Outcomes

The primary outcome was the change in the CTT, that is, the temperature at which a patient will start to develop symptoms, from baseline to week 10 of the treatment period, as determined by provocation testing using TempTest.

Secondary outcomes included the safety of patients treated with omalizumab, the long-term effects of omalizumab on the reduction of trigger thresholds, and the rate of complete and partial responders to omalizumab. Safety was evaluated by recording and monitoring the frequency of treatment-emergent adverse events and serious adverse events. Long-term effects were assessed 8 weeks after the last injection. *Complete response to treatment* was defined as a negative provocation test result for the lowest temperature (4°C), at the end of week 10 after the start of treatment. *Partial response* was defined as an improvement of at least 4°C in the CTT.

Statistical analysis

CUTEX is the first clinical trial with the aim to obtain efficacy and safety data for omalizumab in cold urtacaria. Accordingly, a sample size calculation based on previous data was not possible.

Baseline demographic data and clinical characteristics are expressed as frequency (n) and percentages for sex, median and interquartile range for age, and mean ± SD for body mass index, provocation thresholds, and duration of disease. Continuous variables, that is, changes from baseline in provocation threshold at week 10 (primary end point) as well as CTTs at different time points during the study course, are expressed as means ± SEMs. Categorical data (responder rates) are presented as frequencies (n) and percentages.

The primary end point was analyzed by 1-way ANOVA. In case of a significant result, all 3 treatment arms were compared without correction for multiple testing by using the unpaired Student *t* test. The latter was also applied for the comparison of the CTTs at different time points during the study course. In all analyses with the unpaired Student *t* test, *P* values were chosen from the test performed either for "equal variances assumed" or for "equal variances not assumed," depending on the Levene test for equality of variances. The responder distribution at week 10 was compared between treatment arms with the chi-square test. The statistical program used was IBM SPSS Statistics Version 22, IBM, Ehningen, Germany.

All efficacy analyses were done in the per-protocol population, and safety analyses were performed in all participants who received at least 1 dose of study drug.

REFERENCE

E1. Magerl M, Abajian M, Krause K, Altrichter S, Siebenhaar F, Church MK. An improved Peltier effect-based instrument for critical temperature threshold measurement in cold- and heat-induced urticaria. J Eur Acad Dermatol Venereol 2015;29:2043-5.

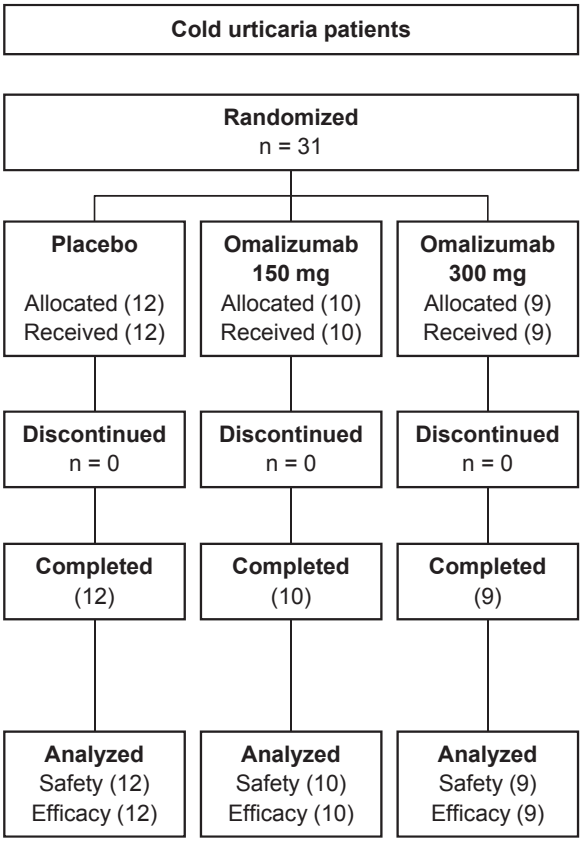

FIG E1. Trial profile.

TABLE E1. Baseline demographic and clinical characteristics of the intention-to-treat population by treatment group

	Patients with cold urticaria			
Characteristic	**All patients (n = 31)**	**Placebo (n = 12)**	**Omalizumab 150 mg (n = 10)**	**Omalizumab 300 mg (n = 9)**
Sex				
Male	8 (26)	3 (25)	3 (30)	2 (22)
Female	23 (74)	9 (75)	7 (70)	7 (78)
Age (y)	45 (32-56)	46 (34-50)	32 (28-63)	46 (39-64)
BMI (kg/m^2)	27.0 ± 4.4	27.0 ± 4.8	26.2 ± 5.1	27.9 ± 3.4
CTT at baseline (°C)	21.5 ± 4.5	20.8 ± 4.3	21.4 ± 5.1	22.4 ± 4.3
Duration of cold urticaria (mo)	80.8 ± 86.6	65.5 ± 76.1	103.4 ± 111.8	76.2 ± 72.5

Data are n (%) for sex, median (interquartile range) for age, or mean ± SD for body mass index, provocation thresholds at baseline, and duration of disease.

TABLE E2. Reported adverse events

Description	Patients with cold urticaria		
	Placebo (n = 12)	Omalizumab 150 mg (n = 10)	Omalizumab 300 mg (n = 9)
Total number of adverse events	35	20	18
Total number of serious adverse events	0	0	0
Patients with any adverse event	9 (75)	7 (70)	7 (78)
Patients with any serious adverse event	0	0	0
Discontinuation due to adverse event	0	0	0
Adverse events observed in more than 1 patient			
Upper respiratory tract infection	4 (33)	2 (20)	2 (22)
Other infections	4 (33)	1 (10)	1 (11)
Headache	1 (8)	2 (20)	2 (22)
Local reaction at injection site	0	2 (20)	0

Adverse events occurring in 5% or more of patients (ie, more than 1 patient) in the placebo or either omalizumab group are listed separately. Data are n or n (%). Adverse events were classified according to *International Classification of Diseases, Tenth Revision* classifications. A patient who reported 2 or more adverse events within the same organ system was counted only once for that term.

TABLE E3. Comparison of complete responders and partial responders or nonresponders

Characteristic	Nonresponder or partial responder (n = 11)	Complete responder (n = 8)
Atopy	1 (9.1%)	5 (62.5%)*
Food allergy	1 (9.1%)	1 (12.5%)
CSU comorbidity	1 (9.1%)	0 (0%)
Presence of other types of CINDU	1 (9.1%)	0 (0%)
Duration of cold urticaria (y), mean ± SEM	9.5 ± 2.5	4.9 ± 2.3

Patients were considered positive for "atopy" if there was a positive history of allergic diseases, eg, allergic rhinoconjunctivitis.
CINDU, Chronic inducible urticaria; *CSU*, chronic spontaneous urticaria.
*$P = .04$, all other parameters showed no statistically significant differences.