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THERAPEUTIC OPTIONS FOR CTLA-4 INSUFFICIENCY

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KEYWORDS: CTLA-4, common variable immunodeficiency, primary immunodeficiency, diagnosis, treatment, abatacept, sirolimus, LRBA, rituximab, HSCT

ABBREVIATIONS USED

AIHA: Autoimmune hemolytic anemia

CNS: Central nervous system

CSF: Cerebrospinal fluid

CVID: Common variable immunodeficiency

CTLA-4: Cytotoxic T lymphocyte-associated antigen-4

DEF6: Differentially expressed in FDCP6 homolog

GFP: Green fluorescent protein

GLILD: Granulomatous-lymphocytic interstitial lung disease

HSCT: Hematopoietic stem cell transplantation

ICOS: Inducible costimulator

IEI: Inborn error of immunity

IPEX: Immune dysregulation, polyendocrinopathy, enteropathy, X-linked

IgRT: Immunoglobulin replacement therapy

ITP: Immune thrombocytopenia

IVIG: Intravenous immunoglobulin

LRBA: Lipopolysaccharide-responsive beige-like anchor protein

MOG: Myelin oligodendrocyte glycoprotein

MRI: Magnetic resonance imaging

PRCA: Pure red cell aplasia

TPO-RA: Thrombopoietin receptor agonist

Treg: Regulatory T

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BACKGROUND: Heterozygous germline mutations in cytotoxic T lymphocyte–associated antigen-4 (CTLA4) impair the immunomodulatory function of regulatory T cells. Affected individuals are prone to life-threatening autoimmune and lymphoproliferative complications. A number of therapeutic options are currently being used with variable effectiveness.

OBJECTIVE: Our aim was to characterize the responsiveness of patients with CTLA-4 insufficiency to specific therapies and provide recommendations for the diagnostic workup and therapy at an organ-specific level.

METHODS: Clinical features, laboratory findings, and response to treatment were reviewed retrospectively in an international cohort of 173 carriers of CTLA4 mutation. Patients were followed between 2014 and 2020 for a total of 2624 months from diagnosis. Clinical manifestations were grouped on the basis of organ-specific involvement. Medication use and response were recorded and evaluated.

RESULTS: Among the 173 CTLA4 mutation carriers, 123 (71%) had been treated for immune complications. Abatacept, rituximab, sirolimus, and corticosteroids ameliorated disease severity, especially in cases of cytopenias and lymphocytic organ infiltration of the gut, lungs, and central nervous system. Immunoglobulin replacement was effective in prevention of infection. Only 4 of 16 patients (25%) with cytopenia who underwent splenectomy had a sustained clinical response. Cure was achieved with stem cell transplantation in 13 of 18 patients (72%). As a result of the aforementioned methods, organspecific treatment pathways were developed. Conclusion: Systemic immunosuppressants and abatacept may provide partial control but require ongoing administration. Allogeneic hematopoietic stem cell transplantation offers a possible cure for patients with CTLA-4 insufficiency. (J Allergy Clin Immunol 2022;149:736-46.)

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Heterozygous germline mutations in cytotoxic T lymphocyte- associated antigen-4 (CTLA4) cause an immune dysregulation syndrome in humans that is termed *CTLA-4 insufficiency*.[1,2] The disease was first described in 2014, and the number of published cases has risen rapidly.[1-3] The diagnosis is made by gene sequencing and study of CTLA-4 function and expression.

Loss-of-function mutations in *CTLA4* result in immune activation by impairing the negative regulation of T-cell activation.[2] The regulatory role of CTLA-4 was first described in *CTLA4* knockout mice, which showed fatal autoimmunity caused by an expansion of activated T cells.[4] Patients with heterozygous *CTLA4* mutations present a similar but less severe phenotype with incomplete penetrance.[1,2]

The phenotype consists of hypogammaglobulinemia associated with recurrent respiratory infections, autoimmune features (cytopenias, (poly)endocrinopathy, and type-1 diabetes), and lymphoproliferation, which can be malignant. Lymphoid or granulomatous infiltrations can be found in the lung, brain, gut, spleen, liver, kidney, and skin. Additionally, *CTLA4* mutation carriers suffer from chronic inflammatory bowel and rheumatic diseases. Currently, therapy focuses on T- or B-cell-specific immunosuppression, including biologics, whereas stem cell transplantation may be curative.[3,5] However, treatment regimens or symptom-specific recommendations are missing.

Here, we present the detailed outcomes of 123 symptomatic carriers of *CTLA4* mutation. On an organ-specific level, we review the effectiveness of the different therapies used. Our therapeutic regimens were driven by literature searches and our retrospective treatment evaluation, aiming for improved treatment options for our patients. We add new pathogenic mutations and demonstrate the need for functional studies of new gene variants in *CTLA4*.

METHODS

PATIENT COHORT

We collected data on a worldwide cohort of 173 *CTLA4* mutation carriers over a 4-year period (July 2014 to September 2017). The data capture reported by Schwab et al[3] ended in September 2016. We continued data collection with a focus on treatment and its outcome. Hence, all 133 individuals in the work of Schwab et al[3] and their allocated identifiers are identical to the first 133 patients of this report (see Table E1 in the Online Repository at www.jacionline.org).

The current cohort consists of 81 families. The individuals were between the ages of 1 and 73 years (88 females and 85 males, witha median age of onset of 13 years and median age of evaluation of 24 years [for details, see Table E1]). Of the entire cohort, 123 individuals (penetrance of71%) had symptoms related to CTLA-4 insufficiency requiring treatment.

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DATA COLLECTION

All contributing physicians were provided with a detailed questionnaire, usually by mail contact. We recorded the mutation carriers' phenotype and their clinical history, including therapy. Within 173 mutation carriers, 54 different mutations were reported. For all 54 mutations, we collected data on pathogenicity from the literature, our own data, and the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) (see Table E1).

For those mutation carriers who were registered before September 2016 (n = 133),[3] we contacted the collaborating physicians for a second time and sent a specific follow-up form to obtain the most recent clinical data and treatment outcome, so that the patient's full clinical history was validated and all treatment relevant data and existing information were updated.

Three individuals were excluded from the analyses: 1 with a large chromosome 2 deletion (2q33.2-2q33.3; including the genes *CD28* and inducible costimulator *[ICOS]),* possibly confounding the phenotype; 1 with the missense variant p.N145S; and 1 with the missense variant p.T207A (located in exon 4 of *CTLA4*). For both missense variants, the results of functional testing (CTLA-4 surface expression and CTLA-4-mediated transendocytosis) were normal.

ETHICS

The study was carried out in accordance with the recommendations of the scientific committee at the University Medical Center of Freiburg for studies involving human subjects. All physicians confirmed that their patients had signed an informed consent under local ethics-approved protocols and in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the University Medical Center of Freiburg (approval nos. 295/13_140782 and 60/18). No financial incentive was provided to the patients or the contributing physicians. Data were reported after having been pseudonymized, and physician-to-physician contact allowed communication of treatment results and advice.

TREATMENT OUTCOME

With regard to the 123 clinically symptomatic individuals, we focused on treatment with immunosuppressants, disease-modifying antirheumatic drugs, biologics, antibiotics, splenectomy, and hematopoietic stem cell transplantation (HSCT). For all drugs, we requested indication, dosage and interval, side effects, and treatment response based on the physician's judgment. A total of 50 unaffected mutation carriers were analyzed regarding their clinical phenotype.

We created groups according to the most affected organ systems (hematologic; hypogammaglobulinemia; and lymphoid, lung, gastrointestinal, neurologic, and skin involvement).

The patient's phenotype determined the organ group; patients affected by multiple organs were part of multiple organ groups independently of whether the manifestations occurred concurrently or consecutively. In fact, immunosuppressive drugs were often administered to alleviate

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multisystemic manifestations and were therefore listed for multiple organ groups despite given only once.

GENERATION OF MANAGEMENT CHARTS

To generate organ-specific diagnosis and treatment flowcharts, we took the following steps, as suggested by the World Health Organization for the generation of consensus documents: (1) we defined 7 groups of the most important clinical manifestations of CTLA4-4 insufficiency; (2) we identified existing guidelines and systematic reviews in the literature for specific symptoms; (3) we generated a draft topic list of opportunities for treatment/ diagnosis improvements in CTLA4-4 insufficiency and created the first draft charts; (4) we performed an internal review (by D.E., B.G., and K.W.) of the draft management charts and revised them accordingly; (5) we called for an external review by all 73 coauthors in an initial contact (via e-mail); (6) 7 coauthors (D.E., B.G., K.W., D.B., P.A., L.A, and T.A.) volunteered to form a task force for the improvement of the management charts; (7) the task force discussed, changed, and revised the management charts; (8) we called for a second external review (via mail) by all 73 coauthors (1 coauthor could not agree on the management protocols and therefore asked to be removed from the list of authors); (9) we performed another internal review for consistency and obtained approval from all coauthors for the final management charts; and (10) the consented management charts were submitted to the peer review process of the *Journal of Allergy and Clinical Immunology.*

Taken together, the charts are based on a literature search, retrospective treatment evaluation, and real-life experience of contributing authors. This process is actually in conformity with the World Health Organization guidelines for development of expert opinion-guided management guidelines with evidence level 4. The charts therefore represent the opinion of an expert panel based on a nonrandomized case series; hence the use of evidence level 4 (of 5) with a recommendation grade of C.

CTLA-4 STAINING AND TRANSENDOCYTOSIS ASSAY

When blood samples from patients carrying variants of uncertain significance in CTLA-4 were available, intracellular CTLA-4 expression and CTLA-4-dependent transendocytosis were evaluated by flow cytometry, as described by Schubert et al.[1] Briefly, primary human $CD4^1$ T cells from patients and controls were purified from PBMCs by negative selection following activation with CD3/CD28 beads (Invitrogen) for 16 hours in the presence of either Chinese hamster ovary cells stably expressing CD80-green fluorescent protein (GFP) or mouse embryonic fibroblasts stably expressing CD80-mScarlet. After 16 hours of incubation, cells were harvested and stained for extracellular markers with anti-CD4-PercPCy5.5 (Invitrogen) and anti- CD45RO-PECy7 (Invitrogen). Following fixation and permeabilization, cells were stained with anti-FOXP3-PE (Invitrogen) and ant.iCTLA-4BV421 (BD Bioscience), and later acquired on a FACS Canto II flow cytometer. Analyses of CTLA-4 expression and CD80-GFP/mScarlet-uptake were evaluated in Tregs gated as $CD4^+CD45RO^+FOXP3^+CTLA4^+$ by using FlowJo 7.6.5 software (TreeStar Inc, Ashland, Ore).

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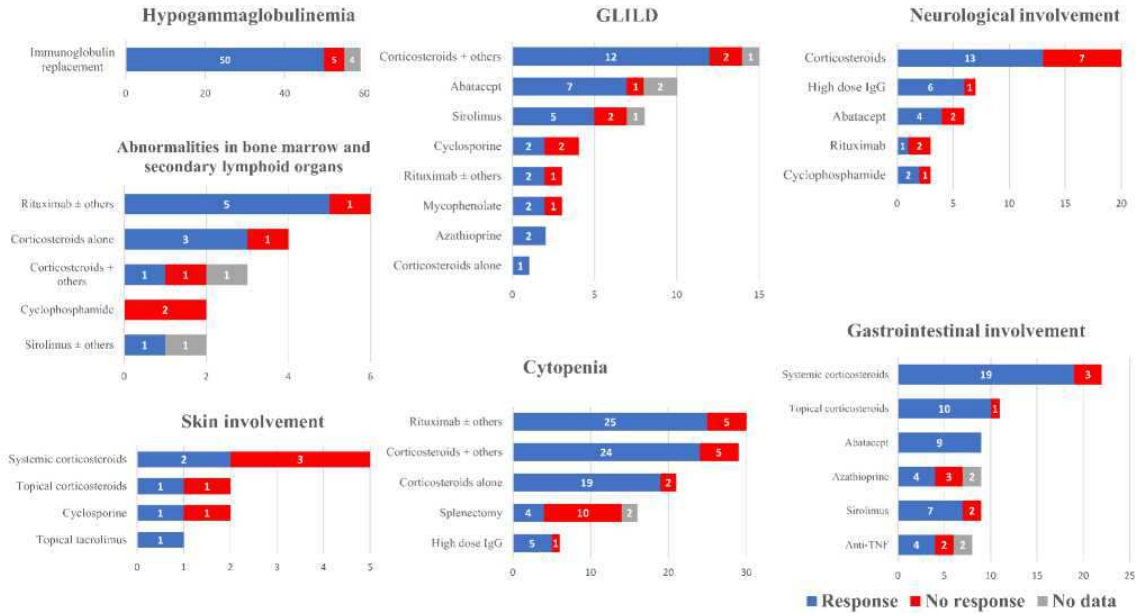
Figure 1. Drug applications in a clinically affected *CTLA4* mutation carrier ordered in organ-specific groups.



STATISTICAL ANALYSIS

All statistics and figures were computed and created by using R (version 3.4.1), Microsoft Excel (2016), or FlowJo 7.6.5 (TreeStar Inc). The drug- related outcome was listed in 7 contingency tables to display how different treatments have produced different outcomes (classified as response, no response, or no data [see Table E2 in the Online Repository at www.jacionline.org]). The Fisher exact test was performed to test for independence as to whether treatment affected the outcome (alpha = 5%; 95% CI = 0.95). The percentage of responses was computed by dividing the number of positive effects by the number of total administrations.

RESULTS

PATIENT COHORT

We are reporting on 173 individuals bearing 54 distinct *CTLA4* mutations. Data on 139 patients have already been published.[3,5-10] Hence, 34 individuals are described here for the first time. The clinical penetrance of the cohort was mutation independent and reached 71% (123 of 173) (see Fig E1 in the Online Repository at www.jacionline.org). The median age at diagnosis was 26 years. At data lock, 18 of 123 symptomatic mutation carriers were younger than 16 years. The clinical penetrance was not a function of age and even decreased to 52% in the cohort of mutation carriers aged 40 years and older, as 23 affected mutation carriers have died at a median age of 24 years. Patients were followed for a total of 47,340 months (median 344 months) since birth and 2,624 months (median 20 months) since molecular diagnosis.

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Treatment details were available for 117 (95%) of the 123 clinically symptomatic patients in our cohort. Most of these patients (111of 123 [90%]) had more than 1 organ system affected, reflecting the complex clinical phenotype of CTLA-4 insufficiency. The number of organ systems involved did not increase significantly at the follow-up visits compared with at the time at diagnosis. We constructed 7 groups with typical manifestations and analyzed their therapy outcomes (Fig 1). Liver involvement, including autoimmune hepatitis, fibrosis, and lymphocytic infiltrations, had been previously underestimated, as it occurred in 18 affected mutation carriers (15%). Regarding the treatment with abatacept (CTLA-4 Fc), we now are reporting on 29 patients (including 13 updated reports from Schwab et al[3]), as detailed in this article.

VARIANTS AND ASSAYS

Among the 34 individuals not previously reported in a publication, we documented 6 novel variants: p.L47P, p.A86fs, p.L133P, p.Q148*, p.Y150X, and p.S172W. We performed functional testing on 6 additional variants (all previously untested): p.A54T, p.M123Ifs*15, p.P136L, p.N145S, p.F179Cfs*29, and p.T207A. We measured CTLA-4 expression levels and CTLA-4-mediated transendocytosis (Fig 2). Regulatory T (Treg) cells with p.A54T, p.P136L, and p.M123Ifs*15 mutations had a reduced CTLA-4 expression, whereas the CTLA-4 expression levels in Treg cells with the p.F179Cfs*29, p.N145S, and p.T207A mutations were comparable to those in healthy controls (Fig 2, *A*). Although Treg cells with the variant p.F179Cfs*29 had normal CTLA-4 expression, the frequency of CD80 uptake in Treg cells was as reduced as in 3 additional deleterious variants (Fig 2, *B).* In contrast, the p.N145S and p.T207A variants showed a normal CD80 uptake and normal CTLA-4 expression (Fig 2, *B* and *C*) and were hence considered benign and not included in our cohort.

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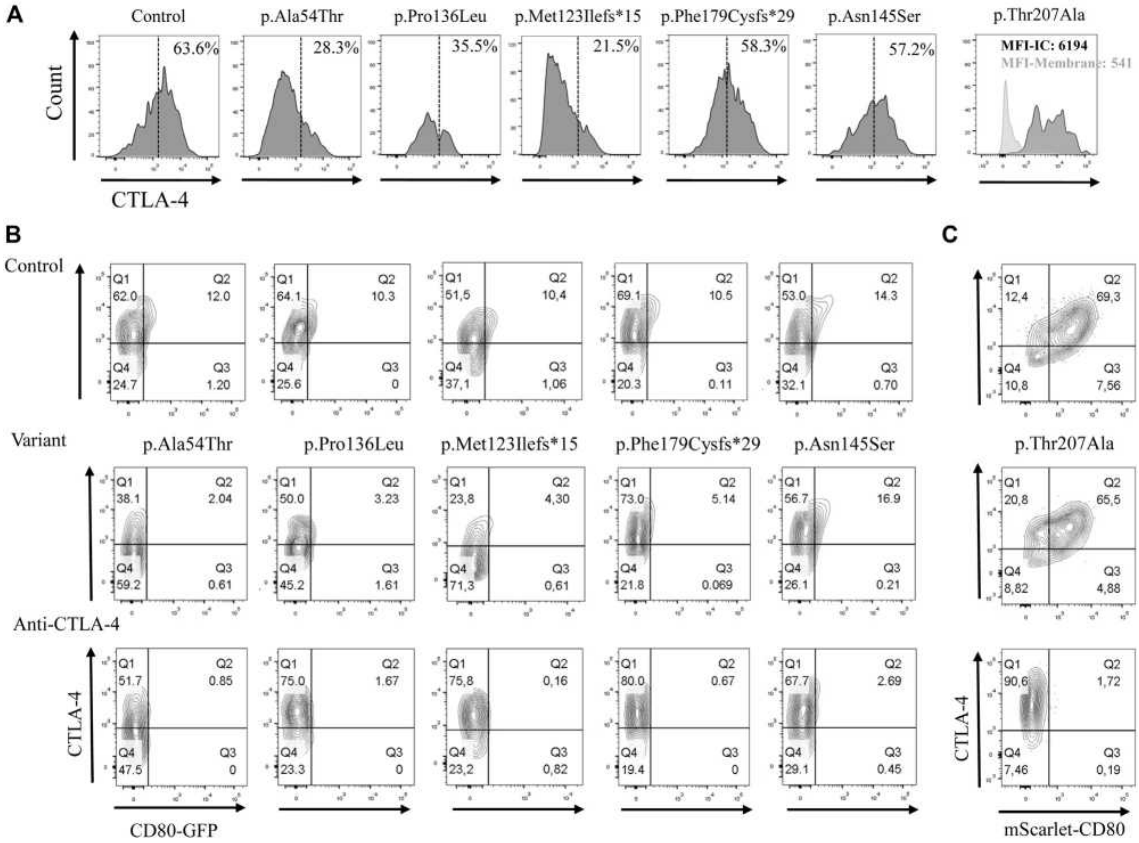
Figure 2. CTLA-4 expression and relative CD80-GFP uptake in *CTLA4* mutation carriers and 1 wild-type control. **A,** Treg cellswith p.A54T, p.P136L, and p.M123Ifs*15showed reduced CTLA-4 expression. The expression levels of CTLA-4 in Treg cells ofthe patients with p.F179Cfs*29, p.N145S, and p.T207A mutationswere com- parableto those in the healthycontrols. **B,** Thefrequencyof CD80-GFP uptakewas reduced in Treg cells with p.A54T, p.P136L, p.M123Ifs*15, and p.F179Cfs*29 mutations. In contrast, p.N145S showed CD80-GFP uptake comparable to that in the healthy control. **C,** The frequency of mouse embryonic fibroblast (MEF)- CD80-Scarlet uptake was comparable to that in the healthy control. The data shown are representative of 2 experiments



TREATMENT

HSCT. Of our 173 patients, 18 underwent HSCT, including 12 who were initially reported by Schwab et al and whose condition has been updated in this article,[3] which already included the 6 patients from Slatter et al.[5] Of these 18 patients, 13 are alive, well, and no longer taking any medications, the only exception being 1 patient who is undergoing immunoglobulin replacement therapy (IgRT) (see Table E3 in the Online Repository at www. jacionline.org). The median time between the age of onset and the transplantation procedure was 6 years. The median followup after the transplantation procedure was 634 days. The indications for HSCT were refractory autoimmune cytopenias, lymphoid infiltration in nonlymphoid organs, severe enteropathy, or malignancy (in decreasing frequency). A total of 5 patients died: 2 of graft-versus-host disease, 1 of acute respiratory distress syndrome, 1 of multiple organ failure, and 1 of diabetic ketoacidosis unrelated to the HSCT.

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Hypogammaglobulinemia. Of the 123 affected patients, 94 (76%) had hypogammaglobulinemia, of whom 63 (76%) experienced recurrent respiratory infections. Of these 63 patients 59 received IgRT (0.4-0.6 g/kg every 3-4 weeks). In 50 (91%) of the 59 patients who received IgRT, susceptibility to respiratory infections clearly improved, as measured by the number of infections (see Table E2, A). Antibiotic prophylaxis was given in 48 (39%) of the 123 cases. The most used antibiotic for prophylaxis was trimethoprim-sulfamethoxazole (in 24 patients) followed by azithromycin (in 6 patients). Penicillin was used prophylactically in 3 patients after splenectomy. In 7 of the 59 patients (11%), side effects of the IgRT such as fever or rash were observed, but they were controlled either by premedication with systemic steroids or by switching to subcutaneous application. In addition to the 59 patients with primary hypogammaglobulinemia, 8 patients in our cohort received IgRT because of secondary loss of antibodies after therapy with rituximab.

Abnormalities in bone marrow and secondary lymphoid organs. Of the 123 affected patients, 90 (73%) developed noninfectious lymphoid disorders. The most common clinical manifestations were lymphoid expansion with splenomegaly (in 69 of 123 patients), lymph node enlargement (in 51 of 123), and hepatosplenomegaly (in 22 of 123). Lymphocytic infiltrations of organs were reported in 66 of 123 patients (54%). Lymphocytic infiltrations and lymphadenopathy with or without hepatomegaly and/or splenomegaly were treated only when associated with organ dysfunction, particularly with autoimmune cytopenias. Seven patients received corticosteroids (5-20 mg per day), with 4 patients (57%) responding favorably. Six patients received rituximab (2-4 X 375 mg/m^2), with 5 positive responses (83%); 2 patients received sirolimus (2-4 mg per day); with a response in 1 of them; and 2 patients had cyclophosphamide (2 X 500 mg/m^2 and 2 X 200 mg/m^2, respectively) without response (see Table E2, B). In 1 patient lymphocytic infiltration in the bone marrow was treated with corticosteroids (10 mg per day) with transient improvement, which was more sustained after the corticosteroids were combined with sirolimus (2 mg per day). Five patients received abatacept because of lung and brain infiltrations and are discussed in the appropriate sections.

Cytopenias. In all, 77 patients (62%) were symptomatic; of these 77 patients, 45 developed immune thrombocytopenia (ITP [formerly known as idiopathic thrombocytopenic purpura]), 43 developed Evans syndrome, 35 developed autoimmune hemolytic anemia (AIHA),17 developed pancytopenia, 7 developed autoimmune neutropenia, and 3 developed pure red cell aplasia (PRCA). A total of 50 patients were treated with corticosteroids (mostly at doses of 0.25-1 mg/kg of prednisolone per day with slow wean), with 43 of them showing at least transient response (89%). A total of 30 patients received rituximab (1-4 X 375 mg/ m^2), of whom 25 (83%) responded; 3 patients received an additional course of rituximab within 2 or 6 months on account of severe cytopenia and responded well; and 6 patients received immunoglobulin at immunomodulatory doses (1 g/kg for 1-2 days), with positive responses in 5 (83%). Of the cohort patients, 16 had a splenectomy (median age 20 years), with only 4 (25%) showing a sustained response (see Table E2, *C).* Abatacept was reported to be helpful in 1 patient with chronic ITP, in 1 patient with chronic AIHA, and in 1 patient with chronic PRCA (125 mg per week subcutaneously). Of the 77 symptomatic patients, 32 (42%) were receiving IgRT before the presentation of autoimmune cytopenia, indicating that IgRT does not protect against this complication in CTLA-4 insufficiency, although that is thought to be the case for patients with common variable immunodeficiency (CVID).[11]

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GLILD. Of the123 affected patients, 44 (36%) had symptomatic granulomatous-lymphocytic interstitial lung disease (GLILD) needing treatment (in 27 patients [61%], the disease was confirmed on biopsy). Symptoms included dyspnea, cough, and exercise intolerance. Bronchiectasis was present in 25 of 123 patients (20%). Therapeutic outcome was typically evaluated radiologically (by high-resolution computer tomography), physically (on the basis of lung function), and clinically by the physicians.

In all, 25 patients received IgRT (0.4-0.8 g/kg every 3-4 weeks), either for low IgG or specifically as part of the treatment for GLILD. A total of 16 patients received corticosteroids (mostly 5-20 mg per day, with 6 patients initially receiving 40-60 mg per day with prolonged tapering) with 13 positive responses (82%). Abatacept (sometimes with a loading dose of 500-1000 mg intravenously and then typically 125 mg per week subcutaneously) was used in 10 patients. Full resolution occurred in 5 patients, and a partial response occurred in an additional 2 patients (70%). Sirolimus (2-4 mg per day) was administered to 8 patients, with resolution of lesions in 5 of them (62%). Rituximab (3-4 X 375 mg/m^2) was given to 3 patients for systemic lymphoproliferation and GLILD. The lung lesions receded but did not resolve in any of the 3 cases. Azathioprine (3 X 50 mg per day) was given to 2 patients, both of whom showed a reduction of interstitial lung disease. Mycophenolate (1000 mg per day) was given to 3 patients and 2 patients showed a reduction of the extent of GLILD (see Table E2, *D).*

Gastrointestinal involvement. Of 123 patients, 74 (60%) had gastrointestinal involvement, 72 with diarrhea. The underlying causes were autoimmune enteropathy (in 21 of 74 patients), inflammatory bowel disease (in 16 of 74), atrophic gastritis (in 14 of 74), and celiac disease (in 3 of 74).

In 33 patients, gastrointestinal involvement was treated with corticosteroids (22 systemic corticosteroids and 11 oral budeso- nide). Systemic corticosteroids alleviated gastrointestinal symptoms in 19 cases temporarily (86%). Dosages varied between 5 and 50 mg per day and were often tapered to 5 mg. Seven patients received single high-dose bolus injections (1000 mg of methylprednisolone) without observable benefit. Of the 11 patients who received oral budesonide (9 mg per day), 10 achieved a good clinical response. Nine patients received abatacept (125 mg per week subcutaneously with a loading dose of 500 mg intravenously in 3 of them). All 9 showed an initial clinical response, but in 2 cases the diarrhea relapsed and abatacept was discontinued, with a 78% response rate. Nine patients received azathioprine (50-150 mg per day), with 4 (44%) showing a positive response. Eight received anti-TNF-a biologics (eg, adalimumab, 20-80 mg per week, or infliximab, 5-10 mg/kg), with 4 positive responses (56%). A total of 9 patients had sirolimus (1-2 mg per day), leading to 7 positive responses (78%) (for details, see Table E2, E).

Neurologic involvement. Of 123 patients, 41 (33%) had neurologic involvement. Of the latter 41 patients, 27 (65%) developed inflammatory cerebral lesions; 15 of the lesions were analyzed by biopsy. A total of 7 patients (17%) were diagnosed with encephalitis.

For the treatment of inflammatory central nervous system (CNS) lesions, 20 patients received corticosteroids (5-50 mg per day with slow wean, with 6 patients receiving methylprednisolone boluses of 1-3 X 1000 mg); 13 of these patients showed a symptomatic clinical response (65%). However, use of second- line medication was necessary on account of unremitting lesions or

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relapses after steroid tapering. Abatacept (a loading dose of 500-750 mg intravenously followed by 125 mg per week subcutaneously) was added to steroids in 6 patients, with 4 patients (66%) showing stabilization of lesions and clinical improvement. Recently, the case of 1 of these patients has been published.[10] High-dose intravenous immunoglobulin (IVIG) (1 g/kg) was added to steroids in 7 patients, with 6 (86%) showing beneficial effects. Rituximab (4 X 375 mg/m^2) was added to steroids in 3 cases, but only 1 patient showed a good response (this patient additionally received cyclophosphamide). Cyclophosphamide (2 X 200 mg/m^2) plus corticosteroids was tried in 3 cases (2 patients received 5 mg per day and 1 received 40 mg per day). Of these 3 patients, 2 showed a good clinical response (both received 5 mg per day) (see Table E2, *F).*

Skin involvement. Skin involvement was present in 61 of 123 patients (50%). We observed 59 cases of recurrent eczema, with 25 of them diagnosed as atopic dermatitis by the treating dermatologist. Additionally, we observed psoriatic skin irritations in 15 patients, alopecia in 14, warts in 11, and vitiligo in 5.

Four patients received systemic corticosteroids (5-20 mg per day): in 1 case because of granulomatous skin infiltration, in 2 cases because of severe psoriasis, and in 1 case because of atopic dermatitis. Of the 16 patients with psoriasis, 3 received methotrexate (7.5-20 mg per week) with good effect and 2 patients received cyclosporine (2-5 mg/kg) for severe infiltration of skin and lung and for concomitant cytopenia. In 1 patient cyclosporine alleviated the skin lesions (see Table E2, G).

DISCUSSION

This study reports the experience of physicians treating a large cohort of symptomatic *CTLA4* mutation carriers. We followed 123 affected *CTLA4* mutation carriers for a total of 2624 patient months. In all, 90% of patients had multiorgan autoimmunity. Abatacept, rituximab, sirolimus, and topical and systemic corticosteroids ameliorated disease severity, especially in cases of lymphocytic organ infiltration. Splenectomy should be avoided, as the response is not sustained. Cure was achieved with stem cell transplantation in 13 of 18 patients (72%). In detail, we propose the following diagnostic workup and treatment protocols for patients with a defect in CTLA-4-mediated biology. Adjustments for pediatric cases must be considered at the sections indicated in treatment protocols.

FUNCTIONAL TESTING OF *CTLA4* VARIANTS

We functionally tested 6 variants, of which only 4 had a reduced CD80 transendocytosis; hence, the impairing effect of the mutation on the biology of CTLA-4 was proved. This highlights the importance of functional testing in cases in which a variant of unknown significance is identified in *CTLA4* in patients with clinical symptoms compatible with CTLA-4 insufficiency, as the differential diagnosis of CTLA-4 insufficiency includes several other monogenic traits for inborn errors of immunity (IEIs), such as the following: lipopolysaccharide-responsive beige-like anchor protein (LRBA) deficiency[12]; immune dysregulation, polyendocrinopathy, enteropathy, X-linked[13]; autoimmune

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lymphoproliferative syndrome[14]; differentially expressed in FDCP6 homolog (DEF6) deficiency[15]; and nuclear factor-κB insufficiency.[16] Interestingly, of these 4 mutations with proven pathogenicity, only 3 had reduced CTLA-4 staining on the surface of Treg cells, indicating that for diagnosing CTLA-4 insufficiency the CTLA-4 surface stain is not as sensitive as CTLA-4-mediated transendocytosis.

HYPOGAMMAGLOBULINEMIA

In CTLA-4 insufficiency, decreased serum levels of immunoglobulins are manifested with recurrent infections, as seen in other IEIs.[17] In CTLA-4 insufficiency, hypogammaglobulinemia is possibly caused by bone marrow infiltration of activated effector T cells, which prevents long-lived plasma cell survival.[1] Moreover, there seems to be an additional bone marrowindependent loss of memory B cells, which is probably caused by a disturbed function of secondary lymphoid organs. Patients with CTLA-4 insufficiency with hypogammaglobulinemia should follow the European consensus protocols for dosing the IgRT (ie, 0.4-0.8 g/kg every 3-4 weeks).[18] In addition, IgRT should be initiated in cases of isolated deficiency of the antibody subclasses IgG1 and IgG2 when associated with infections at values less than 3.0 g/L for IgG1 and less than 2.0 g/L for IgG2 (for adults), respectively (see Management Chart 1 in the Online Repository at www.jacionline.org). In patients with enteropathy, a secondary loss of serum antibodies via the gut should be considered.

ABNORMALITIES IN BONE MARROW AND SECONDARY LYMPHOID ORGANS

Lymphocytic infiltrations in CTLA-4 insufficiency typically involve the spleen and lymph nodes, as well as the gut, lung, skin, liver, kidney, bone marrow, and CNS.[1,3] Additionally, the mucosa-associated lymphoid tissue may proliferate under sustained immune activation and cause gastrointestinal symptoms.[1,3] Benign lymphadenopathy is common in IEIs[19] and in CTLA-4 insufficiency.[3] There is also an increased risk of cancer, especially lymphomas, in patients with CTLA-4-insufficiency.[9] Hepatomegaly, splenomegaly, or regional lymphadenopathy should be carefully monitored, and if malignancy is suspected, a biopsy should be performed.

Our analysis showed that treatment was given only in cases of symptomatic lymphoid infiltrations, especially in the gut, lung, and brain, whereas asymptomatic cases were monitored. We currently advise treating symptomatic lymphoid infiltrations in CTLA-4 insufficiency with corticosteroids, rituximab, or a combination of both. Although histologic evaluations often show a predominant T-cell infiltrate, the B-cell-targeted anti-CD20 treatment with rituximab was clinically very efficient in our analysis. Alternatively, in cases of involvement of organs such as the lung or brain, a mechanistic target of rapamycin inhibitor or abatacept may be considered (see Management Chart 2 in the Online Repository at www.jacionline.org).

CYTOPENIAS

Autoimmune cytopenias in patients with CTLA-4 insufficiency often have a severe, chronic, and relapsing course.[3] This might be caused by autoantibody-mediated peripheral consumption or T-cell-mediated bone marrow output failure. The pathophysiologic cause of the latter is currently the

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subject of intense research. Drug treatment leads to some response in more than 80% of patients. On the basis of our documented outcomes, we suggest a high dose of steroids plus high-dose IVIG as first-line treatment for ITP in CTLA-4 insufficiency, as in CVID.[20] In AIHA, combining corticosteroids with rituximab was more effective than steroids alone, as reported by Birgens et al for AIHA in general.[21] Rituximab also showed efficiency and safety in ITP[21,22] complicating CVID,[20,23] and it should be used in refractory or relapsing cases of any autoimmune cytopenias in the setting of CTLA-4 insufficiency (see Management Chart 3 in the Online Repository at www.jacionline.org). Rituximab helps to eliminate CD80- and CD86-expressing B cells, which activate effector T cells in the setting of CTLA-4 insufficiency. Thrombopoietin receptor agonists (TPO-RAs) are further second-line therapeutic agents for ITP.[24] We documented 4 applications of TPO-RAs in our CTLA-4 cohort, but a satisfying stabilization of platelet count could be reached in only 1 case. Abatacept may be worthwhile for keeping patients with a history of cytopenia (including PRCA) in remission. Splenectomy may improve the platelet count and complete blood count and reduce steroid dependence; however, the patients with CTLA-4 insufficiency in our cohort showed no sustained response, as already published for patients with Evans syndrome[25] and CVID in general.[26] In individual severe cases of cytopenia that are not being controlled even under high doses of steroids, rituximab, or TPO-RAs, HSCT may become necessary at an early stage. HSCT has been described as an effective therapy escalation for patients with CTLA-4 insufficiency by Slatter et al[5] and Schwab et al.[3] A total of 16 patients with CTLA-4-associated cytopenia were treated by HSCT; of these 16 patients, 12 were cured and 4 died.

GLILD

For the diagnosis of GLILD, computed tomography imaging, a 6-minute walk test, lung function testing with diffusion capacity, documentation of the Medical Research Council Dyspnea Scale score, and the exclusion of an infection by bronchoalveolar lavage are recommended. Lung biopsy may be helpful. For treatment, we advise following the British consensus protocols for GLILD in CVID.[27] In summary, we recommend initiating treatment with corticosteroids when patients are symptomatic or/and have abnormal or deteriorating lung function, focusing especially on the diffusion capacity. We start treatment with 0.5 mg/kg per day, but the dose recommendations range up to 2 mg/kg per day.[27] After steroids are tapered to less than 7.5 mg per day within the first 2 months, a computed tomography scan, 6-minute walk test, and lung function testing (including of diffusion capacity) should be performed to document the success of the therapy. In cases of a nonsatisfactory response to steroids, we suggest adding abatacept, rituximab, mycophenolate, or sirolimus as a second- line agent (see Management Chart 4 in the Online Repository at www.jacionline.org). Recently, abatacept was shown to be safe and effective in 10 patients with CVID with GLILD.[28]

However, as GLILD is usually seen in the context of multisystem involvement and all of our 44 patients with GLILD had additional nonpulmonary features, one may argue that (1) the first attempt to treat GLILD with corticosteroid monotherapy is superfluous and (2) abatacept should be preferred over mycophenolate or sirolimus despite the fact that sirolimus has been successful in 1 of our

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cases.[29] Antibiotics may be used for both prophylaxis and treatment of established infections. Antibiotic treatment of acute exacerbations in patients with bronchiectasis is advised to reduce bacterial load and decrease systemic inflammatory mediators. At times, this may require intravenous or inhaled antibiotics.

GASTROINTESTINAL INVOLVEMENT

Therapy for enteropathy in affected carriers of the *CTLA4* mutation depends on the type and severity of gastrointestinal involvement. In cases of gluten sensitivity or lactose intolerance, the respective diets should be followed. In cases of inflammation without known antigen, we recommend a stepwise protocol starting with (1) topical corticosteroids in the form of budesonide (a combination of standard budesonide for the small bowels and retard capsules for the large bowels) or use of oral sulfasalazine; (2) systemic courses of corticosteroids up to 1 mg/kg; (3) abatacept, anti-TNF-a, or sirolimus; or (4) a combination of the aforementioned. A beneficial effect of abatacept on the gastrointestinal symptoms of patients with CTLA-4 insufficiency has been reported before,[3,30,31] and is it therefore recommended as a first-line steroid-sparing agent in patients with CTLA-4 insufficiency (see Management Chart 5 in the Online Repository at www.jacionline.org).

Alternative strategies should include consideration of sirolimus or the anti-a4β7 integrin mAb vedolizumab.[32] The latter was successful in treating the diarrhea in 2 patients. Interestingly, vedolizumab has also proved successful for gastrointestinal adverse effects in anti-CTLA-4 treatment.[33] On the basis of our data, we suggest sirolimus rather than anti-TNF-a as a second-line agent in cases of CTLA-4 insufficiency. In 9 of our patients the gastrointestinal inflammation was so recalcitrant that HSCT was performed.[3,5] Of these 9 patients, 6 were cured and 3 died of HSCT-related causes.

NEUROLOGIC INVOLVEMENT

We defined CTLA-4-associated CNS syndrome as noninfectious neurologic affection caused by intracerebral lesions in *CTLA4* mutation carriers. Some symptoms were caused by secondary bleeding.[3] We recommend performing a magnetic resonance imaging (MRI) of the brain even in asymptomatic mutation carriers and repeating the scanning every 5 years or more frequently in children and when clinically indicated. If lesions are identified, we advise analyzing cerebrospinal fluid (CSF) and searching for autoantibodies. In patients with neurologic symptoms, MRI, lumbar puncture, and serology are mandatory.

CTLA4 mutation carriers typically show extensive white matter lesions with contrast enhancement.[1-3,10] Despite no autoantibodies having been identified in these patients, we recommend excluding anti-myelin oligodendrocyte glycoprotein (antiMOG) antibodies, as patients with anti-MOG antibodies show similar CNS lesions and hence represent the main differential diagnosis.[34] Testing of anti-MOG antibodies in serum is more sensitive than in the CSF.[34] However, we recommend storing a biobank sample (serum and CSF) for possible future novel autoantibody detection, especially as patients undergoing treatment with CTLA-4 inhibitors may develop different types of autoimmune

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encephalitis associated with neuronal surface antibodies (NMDAR and GABAR).[35] For this reason, neuronal surface antibodies in CSF should be considered in patients with CTLA-4 insufficiency with prominent psychiatric symptoms or movement disorder.

In all patients with CTLA-4-associated CNS syndrome, optimization of baseline treatment with abatacept or sirolimus is recommended to control lymphocyte activation. Abatacept showed benefits in 4 of our patients, including in 1 published case patient with regredient lesions.[10] As recommended for other demyelinating CNS disorders,[36] as first-line treatment we suggest high-dose corticosteroids (methylprednisolone pulses [30 mg/kg/ m^2] at a maximum dose of 1 g for 3-5 days). If no rapid clinical response is observed, adding high dose IVIG (1-2 g/kg) appeared to be valuable. Repeated MRI will be necessary to evaluate treatment response at 3 and 6 months following treatment initiation. As a second-line strategy, rituximab or cyclophosphamide should be considered while continuing the first-line therapy, as recommended for autoimmune encephalitis[37] (see Management Chart 6 in the Online Repository at www.jacionline.org). The data are not currently sufficient to recommend 1 regimen over the other.

SKIN INVOLVEMENT

Skin lesions in individuals with CTLA-4 insufficiency are a possible entry port for infections and should be carefully treated or monitored. As skin involvement is often mild to moderate in patients with CTLA-4 insufficiency, skin-specific therapy in CTLA-4 insufficiency should be performed in the context of possible additional organ involvement. As first-line therapy, we suggest topical treatment with corticosteroids and as second-line topical tacrolimus, respectively pimecrolimus for skin lesions in the face. For psoriasis-like lesions we refer to the psoriasis treatment protocols. If systemic treatment is warranted, we suggest considering abatacept, sirolimus, or cyclosporine with or without systemic corticosteroids (see Management Chart 7 in the Online Repository at www.jacionline.org). In 1 of our patients without detectable T_H17 cell infiltration abatacept did not improve the skin condition.

STUDY LIMITATIONS

The retrospective collection of data represents a limitation in our work. It was especially difficult to discriminate retrospectively between an adverse effect of the treatment and the natural course of CTLA-4 insufficiency. Drugs may have been chosen before the diagnosis of CTLA-4 insufficiency was known and therefore influenced treatment decisions. In many cases, insufficient treatment responses have driven further attempts until a sufficient disease control has been established. Differing concomitant steroid regimens make comparison of treatment responses difficult. Although the length of treatment was recorded, the interaction between different drug regimens was not studied. Finally, treatment outcomes are hard to compare in a multicenter approach without defined protocols. Nevertheless, we observed that in the majority of our patients therapeutic success could be achieved. Often, multiple changes of immunosuppressants were needed owing to adverse effects or steroid dependence. Nonetheless, some treated patients were stable over the

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years. In contrast, untreated or insufficiently treated patients developed further CTLA-4-related symptoms, suggesting a progressive natural course of the disease.

PERSONALIZING PRACTICE AND PHENOTYPE-GENOTYPE CORRELATIONS

Our analysis suggests that treatment-refractory CNS involvement, cytopenias, enteropathy, or lung involvement represent ''red flags'' and should trigger early search for a possible stem cell donor. As about 90% of patients with CTLA-4 insufficiency experience multiorgan disease, treating physicians need to combine our treatment recommendations. Other involvement, such as hypogammaglobulinemia, thyroid disease, diabetes, skin involvement or similar conditions, may actually be managed by a more conservative approach. For pediatric patients developing severe symptoms, HSCT should be considered early before manifestation of disease-related organ dysfunction or consequences of long-term immunosuppression. Treatment in pediatric patients should be age-adjusted and should always be planned in consultation with experienced pediatricians.

Regarding the variable penetrance in affected mutation carriers, the timing of treatment and treatment escalation are central questions. A completely unanswered question is, for example, whether one should preemptively treat oligosymptomatic patients (eg, with abatacept). We are not at this point yet, as we do not know whether, for example, the early replacement of CTLA-4-Fc at a sufficient dose may prevent disease onset and/or disease progression and/or worsening. Basically, in an individual with *CTLA4* mutation, abatacept replaces the individual CTLA-4 that is not being produced sufficiently and/or not functioning. Especially in patients with gastrointestinal involvement or lymphoid infiltrations (in the lung and CNS), abatacept has been shown to elicit effective responses.[3,31] Previously, abatacept was successfully used in patients with LRBA deficiency.[38] The same study described T follicular helper cells as a biomarker of disease activity and treatment response.[38] Future studies of abatacept in patients with CTLA-4 insufficiency should examine different doses and aim for a scoring system to measure efficacy similar to that used by Kiykim et al in patients with LRBA.[38] Until further data are available, blood levels of EBV and cytomegalovirus should be carefully monitored while abatacept is being used because loss of control over oncogenic viruses leading to severe virus-associated viremia or neoplasms in selected patients has been described.[3,9] Interestingly, not all of our patients had satisfactory responses to abatacept. Three of our patients became even worse. However, nonresponsiveness may depend on the amount of abatacept given to the patient. Data on long-term application and safety studies for abatacept in this cohort are missing. To this end, the authors have initiated an investigator-driven trial, called ABACHAI (EudraCT no. 2019-000972-40; DRKS no. DRKS00017736).

So far, only HSCT is regarded as a long-term cure for CTLA-4 insufficiency, but because of its treatment-related morbidity and mortality, it requires careful individual consideration. As we understand the increasing burden and complications of CTLA-4 insufficiency, the specific immunomodulatory therapies may just be ''firefighting,'' whereas what is needed is definitive curative HSCT or, in the longer-term, gene therapy to effectively cure our patients. Tesch et al reported on 17 of 24 patients with LRBA deficiency who had a successful outcome with HSCT.[39] Here,

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we have reported on 18 patients with CTLA-4 insufficiency with HSCT, but to our knowledge, at least 6 additional patients with CTLA-4 have since undergone HSCT and a task force to publish an article on HSCT in these patients is in place. Tesch et al associated severe disease activity, lung involvement, age at transplantation, and delayed HSCT with poor outcome.[39] So far, our data have not shown significant differences in outcome by age at HSCT, transplantation year, and time between age of onset and time of transplantation.

In this article, we have published functional data on 4 pathogenic variants but also added 2 variants to the list of most likely benign *CTLA4* sequence variants (p.N145S and p.T207A). Interestingly, so far only patients with variants in exons 1 to 3 have been published. Here, we have reported on 1 carrier who has a variant in exon 4, which codes for the cytoplasmic tail of CTLA-4. The cytoplasmatic tail of CTLA-4 binds to LRBA, which prevents CTLA-4 from lysosomal degradation. Unlike patients with LRBA deficiency, who are generally more severely affected with a phenocopy of CTLA-4 insufficiency, the 11- year-old carrier of the variant in exon 4 had low IgG serum levels but does not currently require immunosuppressive therapy. Functional testing of the variant showed no impairment in CD80 uptake.

Despite the lack of exon 4 mutations, however, no genotypephenotype correlation has been observed. Hence, the type and location of the heterozygous mutation in *CTLA4* may not have a considerable impact on the clinical outcome.[3]

Nonetheless, the incomplete clinical penetrance of CTLA-4 insufficiency suggests that there are factors in addition to the *CTLA4* mutation that are influencing the phenotype. These may be genetic variants, epigenetic modifications, a difference in the microbiota, infectious triggers, or an unknown environmental trigger. It is also conceivable that in different families there are different disease modifiers that may require different treatment protocols. All of the aforementioned issues should be further studied in the future by collecting new cases worldwide to identify disease modifiers and their possible impact on treatment response. Further studies may also indicate which individuals may possibly become affected and may hence need advice on preventive treatment options. As described, treatment in cases of CTLA-4 insufficiency is challenging, and its optimization is an ongoing process. We are still collecting data on more *CTLA4* mutation carriers worldwide for further improvement of patient management and development of additional targeted therapies.

We thank all patients, family members, and physicians who participated in this study.

Clinical implications: The benefits and risks of symptomatic and curative therapies need to be considered when deciding on the best treatment for patients with CTLA-4 insufficiency.

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