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Mepolizumab as a corticosteroid-sparing agent in lymphocytic variant hypereosinophilic syndrome

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Abstract

Background—Mepolizumab, a monoclonal anti-interleukin-5 (IL-5) antibody, is an effective corticosteroid-sparing agent for patients with F/P-negative hypereosinophilic syndrome. Lymphocytic variant hypereosinophilic syndrome (L-HES) is characterized by marked overproduction of IL-5 by dysregulated T-cells.

Objective—To determine whether patients with L-HES respond to mepolizumab in terms of corticosteroid tapering and eosinophil depletion to the same extent as corticosteroid-responsive F/P-negative HES patients with a normal T-cell profile.

Methods—Patients enrolled in the mepolizumab trial were evaluated for L-HES on the basis of T-cell phenotyping and TCR gene rearrangement patterns, and their serum thymus-and-activation-regulated (TARC) chemokine levels were measured. Response to treatment was compared in patient subgroups, based on results of these analyses.

Results—L-HES was diagnosed in 13 of 63 HES patients with complete T-cell assessments. The ability to taper corticosteroids on mepolizumab was similar in patients with L-HES and those with a normal T-cell profile, although a lower proportion of L-HES patients maintained eosinophil levels below 600/μl. Increased serum TARC levels (>1000 pg/ml) had no significant impact on the ability

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to reduce corticosteroid doses, but a lower proportion of patients with elevated TARC achieved eosinophil control on mepolizumab.

Conclusion—Mepolizumab is an effective corticosteroid-sparing agent for patients with L-HES. In some cases however, eosinophil levels remain above 600/ μ l, suggesting incomplete neutralization of overproduced IL-5 or involvement of other eosinophilopoietic factors.

Keywords

CD3⁺CD4⁺; lymphocytic variant HES; clonal T-cell; TARC; IL-5; mepolizumab

Clinical implications

Mepolizumab is an effective corticosteroid-sparing agent for the majority of patients with L-HES and/or increased serum TARC, but fails to maintain eosinophils below 600/ μ l in a subset of such patients.

Introduction

Hypereosinophilic syndromes (HES) are rare disorders, characterized by sustained, moderate-to-severe hypereosinophilia associated with eosinophil-induced tissue/organ damage and dysfunction [1]. The term encompasses very heterogeneous clinical presentations with respect to the nature and severity of eosinophil-mediated complications, overall prognosis, and response to therapy [2,3]. Distinct pathogenic mechanisms have now been identified, allowing for classification of some patients with HES into more homogeneous and “predictable” disease subsets. One of these entities, termed “lymphocytic variant” HES (L-HES), is characterized by over-production of interleukin (IL)-5 by “type 2” T-cells, leading to “reactive” polyclonal eosinophil expansion and activation [4].

Clinically, patients with L-HES often have predominantly cutaneous manifestations, and long-term follow-up has shown malignant transformation of T-cells in several cases. Diagnosis of L-HES currently relies on combined analysis of T-cell phenotypes and T-cell receptor (TCR) gene rearrangement patterns. Early case studies indeed identified monoclonal T-cell subsets with abnormal expression of surface markers (i.e. TCR α/β ⁺CD3⁺CD4⁺ and CD3⁺CD4⁺CD8⁺) that produced high levels of IL-5 *in vitro*, suggesting their involvement in hypereosinophilia [5,6]. Subsequently, other unusual T-cell phenotypes have been reported in association with HES [4,7,8]. Elevated serum levels of TARC have also been described in patients with HES and evidence of IL-5 over-production [9]. Current treatment of L-HES relies mainly on corticosteroids (CS), eventually combined with IFN α ; both agents target eosinophils and cytokine production by the abnormal T-cells [4,10].

Mepolizumab is a monoclonal anti-IL-5 antibody, which binds to free IL-5 and interferes with engagement of the IL-5 receptor on eosinophils. The efficacy of mepolizumab as a CS-sparing agent in patients with CS-responsive, FIP1L1/PDGFRA (F/P)-negative HES was recently demonstrated in the setting of a double-blind placebo-controlled clinical trial [11]. A significantly higher proportion of patients receiving mepolizumab were able to reduce their daily prednisone (PDN) dose, and even to taper off CS completely, compared with those in the placebo arm. In addition, blood eosinophil levels were more tightly controlled in the active treatment arm, despite lower PDN doses, suggesting that mepolizumab efficiently targets eosinophil expansion in patients with HES. Importantly, mepolizumab was well-tolerated with no significant differences in treatment-related adverse events relative to placebo.

Since L-HES is characterized by increased T-cell production of IL-5 and patients with L-HES generally respond to CS, mepolizumab is a tempting alternative to conventional treatment. It is conceivable, however, that a higher dose of mepolizumab would be needed to effectively neutralize the high levels of IL-5 in patients with L-HES. To address this concern, we identified patients with L-HES in the mepolizumab trial, and determined whether they showed CS tapering and eosinophil depletion to the same extent as the patients with a normal T-cell profile. We also investigated whether increased serum TARC concentrations, a possible surrogate marker for the presence of activated type 2 cytokine-producing cells, predicted response to therapy, irrespective of the presence of a clearly defined abnormal T-cell population.

Material and methods

Patients and blood samples

Eighty-five patients with HES were recruited for inclusion in an international, randomized, double-blind, placebo-controlled clinical trial to investigate the efficacy of mepolizumab as a CS-sparing agent in F/P-negative HES (clinical trial MHE100185; Clinicaltrials.gov identifier: NCT00086658). The trial and associated “exploratory biomarker” study was approved by each participating center’s IRB. The clinical characteristics of the cohort are detailed in the original paper reporting the results of the trial [11]. Patients marked their agreement to this biomarker study by ticking a specific box in the consent form. Two sets of blood samples were drawn at baseline, and at weeks 12, 24 and 36 (or at withdrawal) for the biomarker study. The first, serum and PaxGene tubes for measurement of soluble factors in serum and for PCR analysis of T-cell clonality, was sent to a central laboratory for storage at -20°C. The second, an EDTA tube for T-cell phenotyping by flow cytometry, was sent separately to investigators at University of Utah for North American samples and Université Libre de Bruxelles for European samples for processing within 24 hours. Serum, PaxGene, and EDTA tubes were available for 81, 79, and 63 patients, respectively.

MHE100185 study design

The design of the MHE100185 clinical trial has been described in detail elsewhere [11]. Briefly, patients were eligible for enrollment if they fulfilled Chusid’s criteria for HES, were F/P-negative, and if disease was controlled (eosinophil levels and clinical manifestations) by CS-monotherapy, at a daily PDN dose between 20 and 60 mg. After randomization, patients received either intravenous mepolizumab 750 mg or placebo every 4 weeks. At week 1, PDN was progressively tapered according to a pre-defined algorithm, based on blood eosinophil levels and symptoms. The primary end-point of the study was the ability to taper the daily PDN dose to 10 mg or less, for a period of at least 8 weeks. Secondary, *post-hoc* and exploratory end-points addressed more profound and durable PDN tapering, as well as eosinophil control.

Analysis of T-cell surface phenotype

Lymphocyte phenotyping was performed on whole blood within 24 hours by flow cytometry, using FacsCalibur (Brussels) and BD FACScan™ (Utah) (4-color) machines and CellQuest software. The following fluorochrome-coupled antibodies were purchased from Becton Dickinson (same batches for both sites): Quadritest A (CD3-FITC CD8-PE CD45-PerCP CD4-APC), Quadritest B (CD3-FITC CD16/56-PE CD45-PerCP CD19-APC), CD3-PerCP, CD4-APC, TCRαβ-FITC, CD5-PE, CD7-FITC, CD25-PE, CD2-FITC, and HLA-DR-PE. Appropriate control isotypes were used. Staining and acquisition were performed in Brussels and in Utah, and when the study was completed, acquisition files were centralized in Belgium for analysis.

Measurement of serum TARC and IL-5 levels

Frozen serum samples were sent to Brussels for serum TARC measurement, and to Utah for measurement of IL-4 and IL-5. TARC was measured using the R&D TARC ELISA kit, and Th2 cytokines were measured at ARUP Laboratories (Salt Lake City, Utah); measurements were made in duplicate.

Assessment for T-cell clonality

Frozen PaxGene tubes were sent to Bethesda, where TCRgamma (TRG) gene rearrangement studies were performed as described by Greiner et al [12]. PCR was performed using primers that interrogate TRG rearrangements involving all of the known Vg family members, and the Jg1/2, JP1/2 and JP joining segments. To allow for fluorescence detection, each joining region primer was covalently linked to a unique fluorescent dye. The products were analyzed by capillary electrophoresis on an ABI 3130xl Genetic Analyzer, and electropherograms analyzed using GeneMapper software version 3.7 (ABI). This method can detect a clonal population comprising 2-5% of total T-cells, and identifies approximately 95% of all TRG rearrangements occurring in clonal T-cell proliferations.

Diagnosis of lymphocytic variant HES

Diagnosis of L-HES was based on results of T-cell phenotyping and TCR gene rearrangement patterns. The following conditions had to be met: 1) flow cytometry showing a well-circumscribed, phenotypically aberrant subset previously shown to produce Th2 cytokines at cellular level (i.e. CD3⁺CD4⁺), or 2) flow cytometry showing an expanded population of unconventional T-cells (e.g. CD4⁺CD7⁻) previously shown to produce Th2 cytokines *together with* demonstrated (oligo)clonality.

Statistical analysis

Non-parametric comparisons of group means and medians were made using the Mann-Whitney U test. Proportions were compared using Fisher's exact test. A p-value below 0.05 was considered significant.

Results

Identification and characterization of patients with L-HES enrolled in the MHE100185 clinical trial

Analysis of T-cell surface antigens by flow cytometry on peripheral blood from 63 patients enrolled in the mepolizumab trial revealed that 9 patients (14%) had a well-circumscribed CD3⁺CD4⁺ T-cell lineage subset (patients 1-9, Table 1; Figure 1a). As reported in previous studies, the CD3⁺CD4⁺ cells showed negative staining for TCRα/β, intense staining for CD5 compared with the normal CD3⁺CD4⁺ cells in the same sample, and lower overall CD7 expression (supplementary Table S1 and Figure S1). Absolute numbers of CD3⁺CD4⁺ cells at baseline ranged from 15 to 2914 cells/μl. Assessment of T-cell clonality by PCR analysis of TCR Vγ chains showed clonal rearrangement patterns in 7/9 cases and a restricted (oligo)clonal pattern in one patient. Three additional patients had an expanded population of CD3⁺CD4⁺CD7⁻ T-cells (patients 10-12, Table 1; Figure 1b), one with a clonal TCR rearrangement pattern and two with restricted patterns. A distinct CD3⁺CD8⁺CD5^{lo} subset was detected in one patient (patient 13, Table 1; Figure 1c) with a clonal TCR gene rearrangement pattern. Overall, criteria for L-HES were fulfilled in 13/63 (20.6%) patients for whom phenotyping studies were available (P1-P13, Table 1). None of these patients had lymphocytosis at baseline or during the study (at baseline, GM 1886/μl, range 660-4240/μl). Clinically, skin involvement was the most frequent disease complication, especially in patients

with CD3⁺CD4⁺ cells (Table S2). Other manifestations observed in more than half of cases involved the gastrointestinal tract, lungs, and muscles.

In addition to these well-defined and previously reported phenotypes, expanded unconventional T-cell populations were observed in 2 additional cases (CD3⁺CD4⁺CD8^{dim} and CD3⁺CD4^{dim}CD8⁺, with clonal and restricted TCR rearrangement patterns, respectively; patients 14-15, Table 1; Figure 1d-e). In the absence of published evidence that double positive T-cells play a pathogenic role in HES through production of an eosinophilopoietic factor, these patients were classified as idiopathic HES. An extremely elevated proportion of CD3⁺CD4⁺CD25^{hi} T-cells was observed in one patient (patient 16, Table 1; Figure 1f), who was subsequently found to have mycosis fungoides.

TCR gene rearrangement studies were available for 79 patients. In addition to the patients with phenotypically aberrant T-cell lineage subsets described above, a clonal TCR gene rearrangement pattern was revealed by PCR analysis in 3 patients (patients 17-19, Table 1). We were unable to classify patient 17 because T-cell phenotyping was not performed, whereas patient 19 was classified as idiopathic HES on the basis of unremarkable phenotyping studies. Patient 18, who had extremely elevated serum TARC (218,500 pg/ml), was ultimately diagnosed with angioimmunoblastic T-cell lymphoma (AITL).

Mepolizumab trial end-point achievement in patients with L-HES

Of the 13 patients with an established L-HES diagnosis, 7 received mepolizumab and 6 received placebo. L-HES subjects receiving mepolizumab were significantly more likely to maintain a daily PDN dose ≤ 10 mg for at least 24 weeks than the L-HES patients receiving placebo (Figure 2), and had a lower mean daily PDN dose at the end of the study (4.64 mg in the mepolizumab-treated group, versus 28.23 mg in the placebo-treated group [$p=0.014$]). Four of 7 patients in the mepolizumab arm were CS-free at the end of the trial (versus 0/6 in the placebo group, $p=0.07$). These findings were similar to those for the mepolizumab-treated study subjects without L-HES (Table 2; comparisons were made only between patients with available T-cell phenotyping studies, excluding those with lymphoma). L-HES subjects treated with mepolizumab were also more likely than those receiving placebo to achieve an eosinophil count below 600/ μ l for 8 weeks and for the duration of the trial, but were less likely to maintain eosinophil levels below 600/ μ l during the entire trial than mepolizumab-treated subjects without L-HES (71% versus 100%, respectively; $p=0.045$).

Two subjects who received mepolizumab (one with L-HES and one without) failed to achieve the primary end-point. The first patient, with a population of CD3⁺CD4⁺ cells (P5, Table 1), was unable to taper PDN because of increasing eosinophilia associated with recurrence of symptoms, including angioedema and fever. He was considered a non-responder and was withdrawn from the study. In contrast, the second subject, with a normal T-cell profile, was initially unable to taper PDN despite normalization of peripheral eosinophilia, because of sinusitis and seasonal allergies. PDN taper was ultimately achieved and he (she) has been PDN-free for the past 3 years in the open-label extension study (not shown).

In the placebo treatment arm, no significant differences were observed between subjects with and without L-HES with respect to any of the trial end-points.

Identification of patients with increased serum TARC in the MHE100185 trial

Serum TARC levels were measured at baseline and at 12-week intervals in 81 subjects. Baseline, as well as "peak," serum TARC levels were recorded for each patient. Patients were classified as "high TARC" if baseline and/or peak serum TARC was above 1000 pg/ml. Increased TARC levels were recorded on at least one occasion in 33/81 (41%) subjects (Table

3). Of the 33 patients with elevated TARC, 13 had L-HES (patients T1-T13, Table 3), 2 were retrospectively diagnosed with T-cell lymphoma after inclusion in the trial, and 2 had a clonal TCR rearrangement pattern, but did not fulfill criteria for L-HES because phenotyping was either unremarkable or unavailable. The remaining 16 patients were classified as idiopathic HES; 7 had markedly increased serum IgE levels (>1000 KU/L), and 3 others had significantly elevated serum IL-5 levels.

Treatment response in patients with high versus normal serum TARC levels

Response to treatment was compared between “high TARC” patients versus those with TARC levels below 1000 pg/ml (Table 4). In the mepolizumab treatment arm, only the ability to maintain eosinophils below 600/ μ l throughout the study was significantly different between the two groups (11/15 in the “high TARC” group, versus 24/24 in the group with lower TARC levels; $p=0.017$). In the placebo treatment arm, no significant differences were observed using this cut-off level for serum TARC.

Discussion

The current study took advantage of widespread recruitment of patients with steroid-responsive HES for participation in a clinical trial, in order to estimate the proportion of patients with L-HES and/or increased serum TARC values in a large cohort, and to explore responses of patients with T-cell driven disease to highly targeted eosinophil-specific therapy. Diagnosis of L-HES was based on detection of abnormal T-cell phenotypes associated with evidence of clonal T-cell expansion. Demonstration of T-cell clonality alone was not considered sufficient evidence for L-HES, based on recent studies showing clonal TCR rearrangements in high proportions of HES patients, the majority of whom have normal T-cell phenotypes and no evidence of increased IL-5 production [13,14]. The proportion of patients with L-HES included in this clinical trial (13/63) was approximately 20%, which is similar to that reported by Simon et al (16/60 [25%]) in a cohort of HES patients referred mainly to dermatologists [7]. A recent, large retrospective multicenter study, designed to minimize the referral bias of single-center studies, reported 17% patients with L-HES, but the actual proportion with well-documented T-cell phenotype abnormalities was only 8.7% [15]. The relatively high proportion of phenotypically abnormal T-cell subsets observed in our study can be explained by exclusion of patients with FIP1L1/PDGFRA-associated HES from the clinical trial, and by selection of patients who responded to CS-monotherapy, which likely enriched the cohort for L-HES patients compared with more aggressive, presumably myeloproliferative, disease forms.

Results of T-cell analyses on this patient cohort illustrate the heterogeneity within L-HES and how challenging formal diagnosis of this variant has become. In addition to patients with previously well-characterized phenotypically aberrant T-cell subsets, (oligo)clonal TCR rearrangement patterns were observed in some patients with phenotype abnormalities which have not yet been investigated in the setting of HES (e.g. double positive T-cells). Although demonstration of increased IL-5 production at the single-cell level should clarify the possible pathogenic role of these T-cell subsets, this is not within the bounds of routine explorations, and biomarkers of T-cell driven hypereosinophilia, which override phenotypic and functional differences, are desperately needed. Previous studies show that TARC, which is produced by various cell-types in response to IL-4 [17], may be increased in various conditions associated with *in vivo* Th2-cell activation, including L-HES [9,18-21]. We therefore measured serum TARC to identify additional patients with presumed Th2-driven disease in this study, beyond those with clear-cut phenotype abnormalities. Forty percent (33/81) of HES patients in this study had a peak serum TARC level above 1000 pg/ml (Table 3), including all 13 patients with phenotypically proven L-HES although they were under CS, and even when the proportion of aberrant cells was very low (less than 2.5% lymphocytes in 3 cases).

Mepolizumab was an effective CS-sparing agent in patients with L-HES and/or increased serum TARC, with response rates similar to patients with a normal T-cell profile for the primary and more stringent PDN end-points (Table 2). Remarkably, approximately half of the patients were able to taper completely off CS by the end of the study, regardless of their T-cell profile. In contrast, a lower proportion of patients with L-HES and/or serum TARC above 1000 pg/ml durably maintained eosinophil levels below 600/ μ l during mepolizumab therapy (Tables 2 and 4). Viewed differently, the only 4 patients in the trial whose eosinophil levels increased above 600/ μ l despite mepolizumab had increased serum TARC and clear-cut evidence for Th2-mediated disease. Indeed, 2 of them had CD3⁺CD4⁺ L-HES (patients 5 and 6, Table 1), 1 had a clonal TCR rearrangement pattern and extremely elevated serum TARC (patient 17, Table 1), and the fourth patient had increased serum TARC and IgE levels, as well as the highest serum IL-4 level observed in this study (125 pg/ml, not shown) (patient T27, Table 3). For patients 6 and 17, the rise in eosinophilia was easily overcome with low-dose PDN maintenance therapy (<10 mg daily); in these conditions, HES-related clinical manifestations were well-controlled, and eosinophils never exceeded 1000/ μ l (Table S2). In contrast, patients 5 and T27 were truly resistant to mepolizumab, with uncontrolled eosinophilia and recurrent HES-related symptoms as PDN-tapering was attempted, resulting in permanent withdrawal from the study in one case, and HES-related death in the other. Mepolizumab's failure to deplete eosinophils in these cases likely reflects incomplete neutralization of IL-5, due to marked IL-5 overproduction in vivo and/or antibody-inaccessible local production of IL-5; and possibly uninhibited effects of other eosinophilopoietic factors produced by dysregulated T-cells. Although T-cell production of IL-5 was not assessed in the current study, several publications demonstrate that CD3⁺CD4⁺, CD3⁺CD4⁺CD7⁻, and CD3⁺CD8⁺CD5⁻ T-cells from patients with L-HES [4-7,16], and PBMC from patients with increased serum TARC [9], produce more IL-5 in vitro than CD4 T-cells and PBMC from control subjects and other HES patients. Further evidence that increased TARC levels are indicative of a stronger eosinophilopoietic drive is provided in the placebo-treatment arm, wherein a higher proportion of patients with serum TARC above 2000 pg/ml experienced recurrence of hypereosinophilia within 8 weeks during the initial CS-tapering period (90% vs 46%, $p=0.025$, data not shown). The ongoing open-label extension study should clarify the long-term practical implications of these findings, namely the impact on intervals between mepolizumab infusions, as well as the requirement for CS maintenance therapy.

Overall, these results indicate that mepolizumab 1) represents an effective CS-sparing agent for the majority of patients with T-cell driven HES (i.e. L-HES and/or increased serum TARC), similar to other HES patients, but 2) may provide only incomplete disease control as monotherapy in a subset of these patients, whose eosinophil levels remain above 600/ μ l. Given its excellent tolerance and safety profile [11], mepolizumab represents a very tempting alternative to IFN α , the currently preferred but poorly tolerated CS-sparing agent for this HES variant. This study indicates that once mepolizumab is initiated, blood eosinophil levels and clinical status should, nevertheless, be monitored closely as CS doses are reduced for early detection of resistance or requirement of a PDN maintenance dose. Maintenance of low-dose CS may actually be preferable for all patients with L-HES under mepolizumab, because, in contrast to CS and IFN α [10], mepolizumab is not expected to exert inhibitory effects on the pathogenic T-cells involved in L-HES. Indeed, the abnormal T-cells remain detectable in peripheral blood throughout the study (Table S1) and there is reason to believe that both mepolizumab itself [22] and the CS-tapering it enables may increase their production of Th2 cytokines.

Finally, although the 2 patients with T-cell lymphoma were excluded from our analyses, we underscore that neither reached the primary end-point under mepolizumab. The patient with mycosis fungoides presented persistent marked erythroderma requiring continued CS despite effective depletion of blood and skin-infiltrating eosinophils with mepolizumab, whereas the

patient with AITL responded neither in terms of disease manifestations nor eosinophil levels. Both received appropriate therapy for their lymphoma; the first is in remission, and the second died a week later of cardiorespiratory failure, due to heart infiltration by the malignant T-cells. The fact that 2 patients with high serum TARC levels initially diagnosed with HES were ultimately diagnosed with lymphoma suggests that increased serum TARC should prompt careful assessment for underlying lymphoma, both at diagnosis and at regular intervals. Furthermore, it is conceivable that targeted anti-eosinophil strategies may unmask and even accelerate progression of such diseases by enabling CS tapering in some patients, and thereby alleviating some of the pressure on abnormal T-cells. This possibility is of utmost relevance for patients with L-HES, namely CD3⁺CD4⁺ associated disease, as these T-cells may be pre-malignant, and are sensitive to the pro-apoptotic effects of CS. Prolonged follow-up of patients with L-HES on mepolizumab therapy in the open-label extension study should provide some insight into the effects of treatment on numbers and activation status of pathogenic T-cell subsets.

In conclusion, this study shows that HES patients with phenotypically abnormal T cell subsets and/or increased serum TARC levels respond to mepolizumab and are able to taper CS at a rate that is comparable to that of the overall HES population. However, in contrast to patients with normal T-cell profiles, mepolizumab fails to deplete eosinophils in a subset of patients with T-cell driven disease, suggesting that overproduced IL-5 and/or other eosinophilopoietic factors are not (fully) neutralized. Furthermore, CS-tapering on mepolizumab may unmask CS-suppressed T cell disease. Pursuing low-dose CS may therefore be prudent in L-HES patients, in order to target the potentially pre-malignant T-cells, in parallel with eosinophil-directed therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AITL	angioimmunoblastic T-cell lymphoma
CS	corticosteroid
EDTA	ethylenediaminetetraacetic acid
F/P	FIP1-like 1/Platelet derived growth factor receptor alpha fusion
GM	geometric mean
HES	hypereosinophilic syndrome
IFN	interferon
IL	interleukin
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PDGFRA	platelet-derived growth factor receptor alpha
PDN	prednisone
TARC	thymus and activation regulated chemokine
TCR	T-cell receptor

Figure 1. Phenotypically aberrant T-cell subsets detected by flow cytometry on whole blood from patients enrolled in the MHE100185 trial

Phenotypically aberrant T-cell populations are shown within a closed shape (circle, box) on dot plots, or are delimited by a horizontal bar (M1) on histograms. a: CD3⁺CD4⁺ cells (plot gated on total lymphocytes according to FSC/SSC), b: CD3⁺CD4⁺CD7⁻ cells (histogram gated on CD3⁺CD4⁺ lymphocytes), c: CD3⁺CD4⁺(CD8⁺)CD5^{lo/-} (plot gated on CD3⁺CD4⁺ lymphocytes, which are mainly CD8⁺); d: CD3⁺CD4⁺CD8^{dim} (plot gated on CD3⁺ lymphocytes); e: CD3⁺CD4^{dim}CD8⁺ (plot gated on CD3⁺ lymphocytes); f: CD3⁺CD4⁺CD25^{hi} (histogram gated on CD3⁺CD4⁺ lymphocytes)

Figure 2. Superiority of mepolizumab over placebo in patients with L-HES

The response rate of patients with L-HES to mepolizumab versus placebo is shown; white bars represent the mepolizumab treatment arm, and hatched bars represent the placebo treatment arm. PDN: prednisone. * p-values < 0.05, ** p-values < 0.005. Note: for PDN 0 mg until end of study, two-sided p-value is 0.07 and one-sided p-value is 0.049 using Fischer's Exact test.

Table 1

Patients enrolled in the MHE100185 study with an abnormal T-cell profile.

pt.	sex	age	flow cytometry	Absolute count ¹	TCR gene rearr ²	L-HES crit ³	lymph count ⁴	serum IgE ⁵	serum TARC ⁶	Tx arm ⁷	PE ach ⁸
1	F	43	CD3-CD4+	15	restricted	✓	660	195	67170	P	N
2	M	40	CD3-CD4+	57	clonal	✓	4240	9043	31312	P	N
3	F	23	CD3-CD4+	59	clonal	✓	1580	121	15788	P	N
4	F	46	CD3-CD4+	2387	clonal	✓	3410	6945	98511	P	N
5	M	43	CD3-CD4+	200	clonal	✓	1770	12	1488	M	N
6	F	57	CD3-CD4+	326	clonal	✓	1280	255	35463	M	Y
7	F	29	CD3-CD4+	16	poly	✓	3570	28	6720	M	Y
8	F	46	CD3-CD4+	211	clonal	✓	2280	1083	13924	M	Y
9	M	38	CD3-CD4+	2914	clonal	✓	3350	3165	20705	M	Y
10	M	57	CD3-CD4+CD7-	55	restricted	✓	530	2149	7529	P	N
11	M	71	CD3-CD4+CD7-	211	restricted	✓	2650	12	1488	M	Y
12	F	64	CD3-CD4+CD7-	286	clonal	✓	1580	1445	2830	P	Y
13	M	69	CD3+CD8+CD5lo/neg	146	clonal	✓	1850	355	1430	M	Y
14	F	52	CD3-CD4+CD8dim	292	clonal	no	2760	158	2543	M	Y
15	M	68	CD3-CD4dimCD8+	80	restricted	no	2800	2563	513	P	Y
16	M	69	CD3-CD4+CD25hi	155	poly	CTCL	1710	8	19090	M	N
17	M	66	ND	na	clonal	na	840	87	128743	M	Y
18	M	46	ND	na	clonal	AITL	750	58725	218500	M	N
19	F	37	CD4/CD8 ratio <1	na	clonal	no	1450	11	1398	M	Y

This table includes all patients in whom T-cell phenotyping and/or PCR for TCR rearrangements showed abnormal results.

¹ absolute counts of phenotypically abnormal T-cell subsets per microliter, at baseline; for patients 12 & 13 values are at week 12 (not available or uninterpretable at baseline)

² TCR gene rearrangement pattern at baseline, except patient 7 at week 12; poly indicates polyclonal

³ indicates whether diagnostic criteria for L-HES are met; revised diagnosis of lymphoma is indicated: CTCL cutaneous T-cell lymphoma, AITL angioimmunoblastic T-cell lymphoma, na not applicable because T-cell phenotyping not performed

⁴ absolute lymphocyte count per microliter at baseline, except patients 12 & 13 for whom values are at week 12

⁵ baseline serum IgE levels in KU/L; upper limit for normal values: 114 KU/L

⁶ peak serum TARC level in pg/ml

⁷ treatment arm: P placebo; M mepolizumab

⁸ indicates whether patient achieved primary end-point of the trial; i.e. daily prednisone dose ≤ 10 mg for ≥ 8 weeks

Table 2

Treatment responses in patients with L-HES versus a normal* T-cell profile.

	Mepolizumab			Placebo		
	L-HES (n=7)	Others (n=24)	P value	L-HES (n=6)	Others (n=25)	P value
PDN ≤10mg for ≥ 8 wks (PE)	6 (86%)	23 (96%)	0.41	1 (17%)	10 (40%)	0.38
PDN ≤10mg for ≥ 24 wks	6 (86%)	13 (54%)	0.2	0	3 (12%)	1
Off PDN until study end	4 (57%)	12 (50%)	1	0	0	-
Eos <600/μl for ≥8 wks	6 (86%)	24 (100%)	0.23	1 (17%)	13 (52%)	0.18
Eos <600/μl until study end	5 (71%)	24 (100%)	0.045	0	3 (12%)	1

* normal T-cell profile on the basis of flow cytometry and TCR gene rearrangements.

PDN: prednisone; PE: primary end-point; wks: weeks

Table 3

Patients with increased serum TARC values.

pt.	BL TARC ¹	peak TARC ¹	flow cytometry	TCR rearr	serum IgE ²	serum IL-5 ³	Diagnosis ⁴
<u>Patients with abnormal T-cell phenotype subset and/or T-cell clonality</u>							
T1	1948	67170	CD3-CD4+	restricted	195	<7.8 (122.9)	L-HES (P1)
T2	31312	31312	CD3-CD4+	clonal	9195	43.4	L-HES (P2)
T3	15788	15788	CD3-CD4+	clonal	121	10.6	L-HES (P3)
T4	74895	98511	CD3-CD4+	clonal	7215	<7.8 (8.4)	L-HES (P4)
T5	1038	1488	CD3-CD4+	clonal	23	<7.8	L-HES (P5)
T6	7020	35463	CD3-CD4+	clonal	255	178.5	L-HES (P6)
T7	ND	6720	CD3-CD4+	poly	47	<7.8	L-HES (P7)
T8	5559	13924	CD3-CD4+	clonal	1083	139.6	L-HES (P8)
T9	137	20705	CD3-CD4+	clonal	4032	<7.8	L-HES (P9)
T10	858	7529	CD4+CD7-	restricted	2149	<7.8	L-HES (P10)
T11	849	1488	CD4+CD7-	restricted	150	<7.8	L-HES (P11)
T12	416	2830	CD4+CD7-	clonal	1445	<7.8 (32.8)	L-HES (P12)
T13	390	1430	CD3+CD8+CD5lo/neg	clonal	424	<7.8	L-HES (P13)
T14	6200	19090	CD3+CD4+CD25hi	poly	12	<7.8	CTCL (P16)
T15	32618	218500	ND	clonal	71540	<7.8	AITL (P18)
T16	583	2543	CD3+CD4+CD8dim	clonal	158	<7.8	L-HES (P14)
T17	1862	128743	ND	clonal	124	<7.8	L-HES (P17)
<u>Patients with no overt T-cell abnormalities</u>							
T18	1035	1035	ND	poly	101	<7.8	L-HES
T19	1049	1770	nl	poly	1115	<7.8	L-HES
T20	229	1741	nl	poly	170	<7.8 (78)	L-HES
T21	2311	2311	CD4/CD8: 6	poly	44	177.4	L-HES
T22	169	1930	CD4/CD8: 6.8	poly	29	<7.8 (302.5)	L-HES
T23	726	1009	nl	poly	302	<7.8	L-HES
T24	481	1398	CD4/CD8 <1	clonal	34	<7.8	L-HES
T25	557	6790	CD4/CD8: 8	poly	2495	<7.8	L-HES
T26	ND	1023	nl	poly	214	15	L-HES
T27 ⁵	206	10624	ND	poly	2160	11.6	L-HES

pt.	BL TARC ¹	peak TARC ¹	flow cytometry	TCR rearr	serum IgE ²	serum IL-5 ³	Diagnosis ⁴
T28	2040	6470	CD4/CD8: 5.9	restricted	116	<7.8 (9.9)	I-HES
T29	1619	35413	nl	poly	3122	<7.8	I-HES
T30	818	1492	nl	poly	49	23	I-HES
T31	ND	2721	nl	poly	1700	<7.8	I-HES
T32	7330	9130	ND	poly	10740	<7.8	I-HES
T33	303	1208	ND	poly	17185	<7.8 (15.2)	I-HES

This table includes all patients in whom peak serum TARC was above 1000 pg/ml.

¹ Both baseline (BL) and peak serum TARC levels are shown, expressed in pg/ml.

² Serum IgE is maximum level recorded during the study, in KU/L; normal value < 114.

³ Serum IL-5 is baseline level in pg/ml; normal value < 7.8; values between parentheses are maximum levels recorded during the study in patients in the placebo arm (mepolizumab interferes with serum IL-5 measurements [22]).

⁴ Patient numbers P1, P2, etc... refer to patient numbers in Table 1.

⁵ T27 died of cardiac arrest during the trial (study day 110).

ND: not done. nl: normal. I-HES: idiopathic HES.

Table 4

Treatment responses in HES patients with peak serum TARC levels above versus below 1000 pg/ml.

	Mepolizumab		P value	Placebo		P value
	TARC > 1000 (n=15)	TARC < 1000 (n=24)		TARC > 1000 (n=16)	TARC < 1000 (n=22)	
PDN ≤ 10mg for ≥ 8 wks (PE)	13 (87%)	21 (88%)	1	4 (25%)	11 (50%)	0.18
PDN ≤ 10mg for ≥ 24 wks	8 (53%)	14 (58%)	1	2 (13%)	2 (9%)	1
Off PDN until study end	5 (33%)	13 (54%)	0.32	1 (6%)	0	0.42
Eos < 600/μl for ≥ 8 wks	14 (93%)	23 (96%)	1	4 (25%)	12 (55%)	0.1
Eos < 600/μl until study end	11 (73%)	24 (100%)	0.017	1 (6%)	3 (14%)	0.62