both IL-33 and IgE together with oral allergen immunotherapy may facilitate the development of sustained tolerance by removing both pathways of mast cell-mediated suppression of Treg generation.

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AUTHOR CONTRIBUTIONS

SB performed experiments and analyzed data. LT performed DO11.10 T-cell transfer experiments. CB contributed to experimental design. SB and CB wrote the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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IgE multiplex testing in house dust mite allergy is utile, and sensitivity is comparable to extract-based singleplex testing

To the Editor,

An individualized diagnostic approach determining molecular sensitization patterns of house dust mite (HDM) allergic patients may help to identify best eligible patients for allergen immunotherapy, as modern HDM immunotherapy preparations are usually standardized to the major allergens Der p 1, Der f 1, Der p 2 and Der f 2.[1] However, data on the reliability of molecular HDM allergy diagnosis using commercially available assays are limited.

We aimed to investigate the overall sensitivity of molecular HDM allergy diagnosis compared to extract-based IgE testing using the singleplex assay ImmunoCAP (detecting Der p 1, 2, 10 and 23), the multiplex assay ImmunoCAP ISAC (detecting Der p 1, 2 and 10) and the newly available multiplex platform, Allergy Explorer (ALEX) versions 1 and 2 (version 2 detecting Der p 1, 2, 5, 7, 10, 11, 20, 21 and 23).

Initially, we searched our database for patients with positive skin prick tests to HDM. Data between January 1, 2005, and December 31, 2018, were analysed to determine sensitization rates to the two major species of house dust mite, Dermatophagoides pteronyssinus (D.p.) and Dermatophagoides farinae (D.f.), in Austria. In total, 28 572

TABLE 1 Overall sensitivity of molecular test methods increased with higher sIgE to D.p. extract

sIgE to D.p. extract	n	ISAC (%)	*P*-value	ImmunoCAP molecular (%)	*P*-value	ALEX	*P*-value	ALEX^2	*P*-value
Overall	215	88.8	<.001	93.0	<.001	93.5	<.001	94.9	.001
≥0.7 kU/L	204	89.7	<.001	94.6	.001	94.6	.001	95.6	.004
≥1.0 kU/L	186	93.6	<.001	96.2	.016	96.8	.031	97.3	.063
≥3.5 kU/L	141	97.9	.250	99.3	1.000	99.3	1.000	99.3	1.000

Note: Sensitivity of the four molecular assays tested increased with sIgE levels to D.p. extract. In the case of sIgE levels ≥ 1.0 kU/L, ALEX^2 and in the case of sIgE levels ≥ 3.5 kU/L, all molecular assays performed statistically equal to extract-based diagnosis. All *P*-values listed are direct comparisons to extract-based singleplex diagnosis using ImmunoCAP.

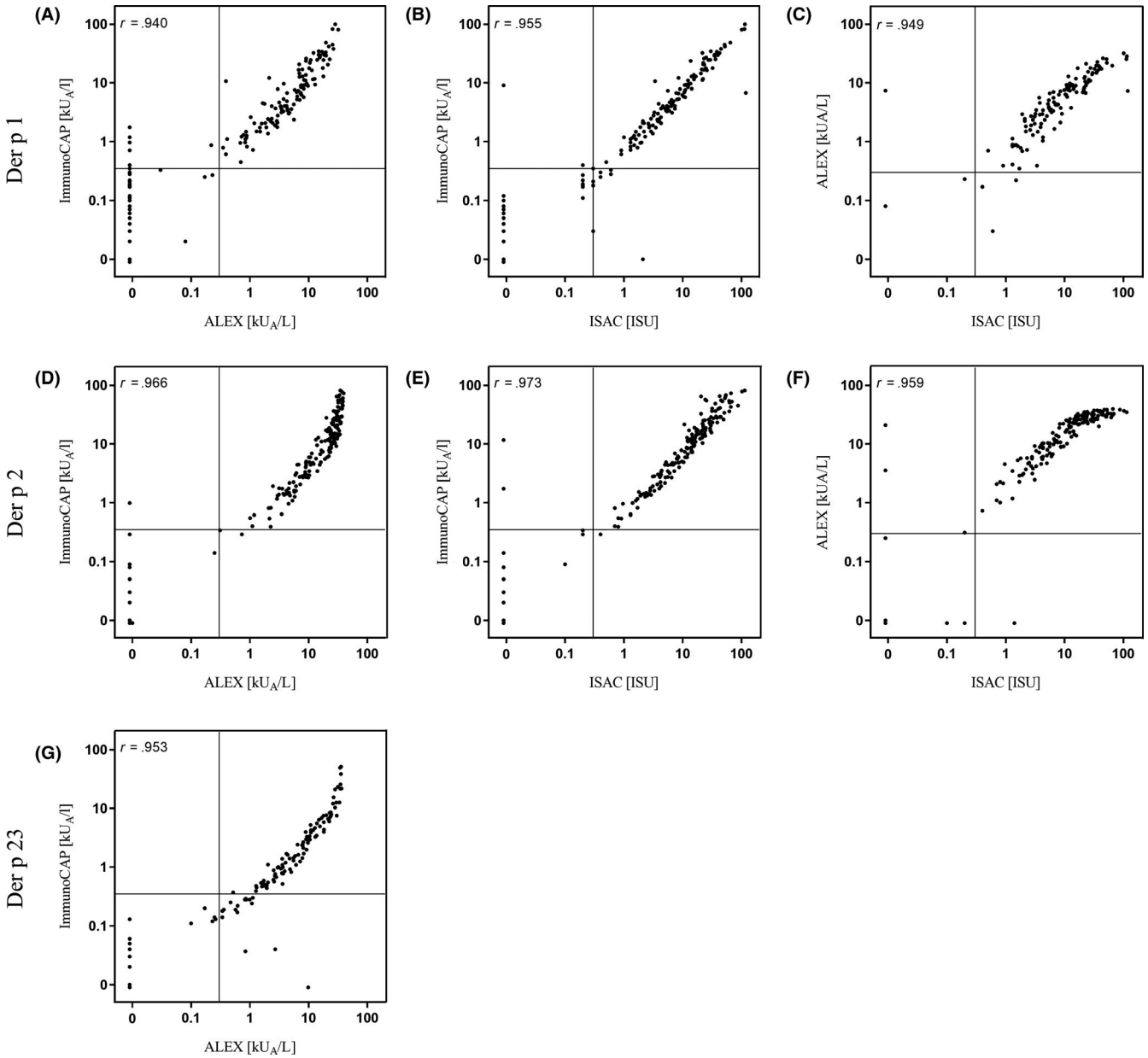

FIGURE 1 High correlation of the molecular allergy test systems. Molecular test systems correlated strongly with Spearman's rho ranging between 0.940 and 0.955 (Der p 1), between 0.959 and 0.973 (Der p 2), and 0.953 (Der p 23), ($P < .001$). Due to the low number of Der p 10 sensitizations, correlations were not calculated for Der p 10

patients had positive skin tests to D.p. and/or D.f. Of these, 23 930 (83.8%) had positive skin prick tests to both, and 3,212 (11.2%) and 1430 (5.0%) were mono-sensitized to D.p. and D.f., respectively. To analyse the different diagnostic methods, sera of 215 HDM allergic patients with unequivocal history of HDM allergy, a positive skin prick test and detectable (≥0.35 kU/L) sIgE to D.p. extract were investigated. Patients were solely sensitized to HDM (defined by sIgE determination and skin prick testing with 7 and 14 inhalant allergens, respectively). For detailed explanation of methods and statistical analysis, see File S1. For demographic and clinical data of the study population, see Table S1.

Overall sensitivity of molecular allergy diagnosis (defined by a positive test reaction to at least one molecular allergen) was lower compared to singleplex extract-based testing, and it usually increased the more house dust mite allergens were available. Overall sensitivity of ISAC, molecular-based ImmunoCAP, ALEX and ALEX^2 was 88.8%, 93.0%, 93.5% and 94.9%, respectively. Results of the molecular-based ImmunoCAP, ALEX and ALEX^2 did not differ significantly, whereas sensitivity of the ISAC was lower compared to ALEX and ALEX^2 (P = .006 and P < .001) as well as to the molecular-based ImmunoCAP (P = .022). This was mainly due to the unavailability of Der p 23: omission of Der p 23 using ImmunoCAP, ALEX and ALEX^2 resulted in a lower overall sensitivity of 87.9%, 88.4% and 90.2%, respectively, which were all similar to the 88.8% of the ISAC (P = .392).

Overall sensitivity of the molecular test systems was clearly correlated with sIgE levels to D.p.: the higher the levels, the better the sensitivity of molecular testing (Table 1).

In our study population, three allergens, Der p 1, 2 and 23, constituted major allergens, with sensitization rates of 55.3%, 77.7% and 54.0%, respectively, whereas all other allergens were minor allergens. Mono-sensitization to Der p 2 was most frequently observed as 21.4% of patients were solely sensitized to Der p 2, followed by 10.7% who were mono-sensitized to Der p 1; 4.7% were solely sensitized to Der p 23. A mere 0.5% of patients were mono-sensitized to Der p 10 and 20, respectively. Importantly, we did not observe mono-sensitization to Der p 5, 7, 11 or 21, indicating that these allergens do not increase sensitivity of the test panel. Using the ImmunoCAP, sensitization rates to Der p 1, 2 and 23 were similar with 58.1%, 77.2% and 46.5%, respectively. Mono-sensitization to Der p 23 was observed in 4.7%, which was identical to the observed rate with the ALEX^2. The sensitization pattern to nine molecular allergens tested with ALEX^2 is depicted in Figure S1. Interestingly, all molecular test systems correlated strongly (Figure 1).

Overall sensitivity of the molecular test platforms investigated was good, ranging from 88.8% to 94.9%. However, even with the best method, 11 out of 215 (5.1%) sera were negative for the nine molecular allergens investigated. Following reasons may explain the lower sensitivity: although nine molecular allergens have been tested, this could still be insufficient, as 30 D.p. molecular allergens have been described so far (retrieved from www.allergen.org, January 26, 2020). Several years ago, it was reported that using a combination of Der p 1 and 2 could detect at least 97% of D.p. allergic patients in Europe,[2] whereas more recent data do not support this observation.[3,4] Besides Der p 1 and 2, Der p 23 is the third major HDM allergen with (mono-) sensitization rates in our study population of 4.7% and 54.0%, respectively, which is similar to previously reported rates between 4.2% and 5.3% for mono-sensitization and between 46.5% and 75.8% of HDM patients sensitized to Der p 23.[5-7] This makes Der p 23 indispensable for diagnosis and explains why all molecular test systems including Der p 23 had a higher sensitivity. In our study, additional testing with Der p 10 and 20 at least slightly increased sensitivity, whereas Der p 5, 7, 11 and 21 did not. Therefore, it would be crucial to add only clinically relevant molecular allergens to a multiplex test panel in the future.

Technical issues could be another reason why modern molecular allergy diagnosis cannot detect all HDM allergic patients. Compared to singleplex assays, sensitivity of multiplex test systems can be decreased in patients with low sIgE levels due to higher limits of detection, higher coefficients of variation and a potential inhibition by antigen-specific IgG.[8] We could clearly show that sensitivity of molecular assays was impaired in patients with low sIgE levels. It should be mentioned that our study population reflected an unbiased random sample out of daily practice, with low (≤1.0 kU/L) sIgE to D.p. in 13.5% of patients. Under optimal conditions, namely in patients with sIgE levels <3.5 kU/L, sensitivities of the molecular test systems were very high, ranging from 97.9% to 99.3%. The newest multiplex assay, ALEX^2, performed statistically equal to extract-based diagnosis in patients with sIgE levels >1.0 kU/L with a sensitivity of 97.3%.

Taken together, modern multiplex testing is an individualized diagnostic approach determining sensitization patterns of HDM allergic patients, which may help to identify best eligible patients for allergen immunotherapy. Sensitivity of up-to-date multiplex systems is now comparable to extract-based testing. In patients with low sIgE levels, however, additional singleplex extract-based testing or prick testing may be necessary.

CONFLICTS OF INTEREST

GJ Sturm reports consulting and lecture fees from Novartis, Bencard, Stallergenes, HAL, Allergopharma and Mylan outside of the submitted work. U Cerpes reports fees from Mylan outside of the submitted work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Exon 8 *KIT* mutation and pulmonary eosinophilia

To the Editor,

The c-*KIT* proto-oncogene encodes for KIT, a tyrosine kinase receptor essential for mast cell development.[1] Mutations affecting this protein are associated with hematological malignancies such as leukemias, hypereosinophilic syndromes (HES), and systemic mastocytosis.[2] *KIT* mutations in patients with pulmonary eosinophilic disorders have not been described. We present three cases of eosinophilic lung disorders harboring identical c-*KIT* exon-8 mutations in patients referred to a combined pulmonary-hemato-oncology eosinophilic disorders clinic. Investigations for parasitosis, vasculitis, sarcoidosis, hypersensitivity pneumonitis, and allergic bronchopulmonary aspergillosis were negative. All subjects provided signed consent to use their sputum and blood samples for biomarker discovery.

Case 1: A 20-year-old woman, who had recently taken up marijuana smoking, was admitted to the hospital with a one month history of fevers, night sweats, productive cough, pleuritic chest pain, fifteen pound weight loss, and peripheral blood eosinophilia (5.7×10^9/L). Tryptase level was normal (4.8 μg/L) and total IgE level was elevated (385 KIU/L). The symptoms and eosinophilia resolved spontaneously during a period of abstinence from smoking marijuana and returned one month later with a peak eosinophil count of 9.0×10^9/L upon resumption of smoking marijuana. Thoracic CT imaging demonstrated bilateral multifocal groundglass opacities and bronchoalveolar lavage identified an eosinophilia of 12%. The symptoms and the eosinophilia resolved after two weeks of oral prednisone 50 mg daily. Bone marrow tests to assess for myeloproliferative and lymphoid disorders included G-banding cytogenetic analysis, molecular studies assessing for *PDGFRA* and *PDGFRB* gene rearrangements, *JAK2* mutations, *BCR-ABL* translocations, clonal T-cell receptor gene rearrangements and flow cytometric analysis for abnormal T-cell populations- and clonal B-cells yielded normal results. The bone marrow morphological assessment identified increased eosinophils without increased myeloblasts. Reverse transcription polymerase chain reaction identified a *KIT* c.1232_1346del(p.Thr411fs) mutation which results in a complete deletion of exon-8 encoding the extracellular domain of the receptor. Two further exacerbations of respiratory symptoms occurred one month apart, both associated with resumption of marijuana use and elevated eosinophil counts of 3.1×10^9/L and 1.1×10^9/L, respectively. The patient was treated with prednisone 15 mg daily and hydroxyurea 500 mg daily; the symptoms, peripheral blood/sputum eosinophilia and chest radiographic abnormalities resolved. Both medications were discontinued after two months of complete abstinence from smoking marijuana.

Case 2: A 66-year-old woman was referred with a 3 month history of cough, shortness of breath, sinus congestion, and a peak eosinophil blood count of 4.6×10^9/L following an innocuous upper respiratory tract infection. The patient had recently switched from smoking cigarettes to e-cigarettes. Total serum IgE was elevated (1146 KIU/L) and