**Exploring biomarkers to predict clinical improvement of Atopic Dermatitis in patients treated with dupilumab (B-PAD study)**

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**Supplementary methods**

**Patients**

This exploratory study is a multi-center, prospective, observational study in which samples/information were obtained in Japan, and was basically carried out under real-world standard treatment guidelines. We enrolled 131 subjects from 19 medical facilities joining a consortium. Excluding cases in which consent to participate was withdrawn and those that were ineligible for inclusion, the final number of subjects for analysis was 124, and the number of subjects for efficacy analysis was 110 (Figure S1). The patients were required to discontinue oral immunosuppressive drugs (cyclosporine), oral steroids, or phototherapy at least 4 weeks (wks) prior to the start of injections of dupilumab. The patients were also required to have no previous experience of dupilumab treatment. They were to be at least 18 years of age, have moderate to severe AD with Eczema Area and Severity Index (EASI) ≥16, Investigator’s Global Assessment (IGA) ≥3, and body surface area ≥10%, and be individuals for whom topical treatment of steroids provided inadequate control or was medically inadvisable, and had suffered chronic AD for at least 3 years before the start of this study. The use of systemic steroids, systemic calcineurin inhibitors, and phototherapy was not allowed after the initiation of dupilumab. However, the continued use of topical steroids, topical calcineurin inhibitors, topical moisturizers, and oral antihistamines used at baseline was allowed. Change of topical drugs to more potent ones was not allowed. Moreover, the use of ocular, intranasal, or inhalant steroids and calcineurin inhibitors was allowed throughout the study, as was the use of anti-histamine drugs. Subjects were set to receive subcutaneous injections of dupilumab (initial dose 600 mg, then 300 mg) biweekly for 16 wks. All investigators involved in this study carried it out in accordance with the latest versions of the Declaration of Helsinki and “Ethical Guidelines for Medical and Health Research involving Human Subjects” of the Ministry of Health, Labour and Welfare, Japan. The study protocol was approved by the Clinical Research Network Fukuoka Certified Review Board (CRB7180004). This study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN000037307).

**Evaluation of clinical outcomes and biomarkers**

Objective clinical findings were evaluated by EASI. Subjective symptoms were assessed by PROs such as Patient-Oriented Eczema Measure (POEM) and Numerical Rating Scale for pruritus (pruritus-NRS) (peak pruritus in the past 24 hours) (Figure S2). Patients were also requested to complete uncomfortable skin-NRS (0: no discomfort, 10: worst discomfort imaginable) and treatment satisfaction-NRS (0: not satisfied at all, 10: very satisfied) (Figure S2). We also measured 19 biomarkers including eosinophil count, LDH, total IgE, soluble IL-2R, CC chemokine ligand (CCL)17/Thymus and activation-regulated chemokine (TARC), CCL18, CCL22, CCL26, CCL27, IL-13, IL-22, IL-24, IL-25, IL-31, IL-33, Thymic stromal lymphopoietin (TSLP), periostin, squamous cell carcinoma antigen 2 (SCCA2), and endothelin-1 (ET-1). The evaluation of clinical outcomes and biomarker sampling were performed on the day of initiating injections of dupilumab and at 2, 4, 8, and 16 weeks (w) of dupilumab treatment (Figure S3).

**Primary and secondary endpoints**

This was an exploratory clinical study to determine which biomarkers were associated with clinical improvement. The primary endpoint was the association between baseline levels of 19 biomarkers and % change from baseline of EASI at 16 w of dupilumab treatment.

Secondary endpoints were (1) the association between baseline levels of 19 biomarkers and % change from baseline of POEM at 16 w, (2) the association between baseline levels of 19 biomarkers and % change from baseline of pruritus-NRS at 16 w, (3) the association between baseline levels of 19 biomarkers and % change from baseline of uncomfortable skin-NRS at 16 w, and (4) the association between baseline levels of 19 biomarkers and % change from baseline of treatment satisfaction-NRS at 16 w.

**Statistical analysis**

We used linear regression models to investigate the associations of each biomarker with the primary endpoint and secondary endpoints, adjusting for sex and age. LDH and TARC were log-transformed since residuals were not normally distributed. All statistical analyses were performed using Stata 17.0 (Stata Corp., College Station, TX, USA). The two-sided significance level for all tests was P < 0.05.

**Supplementary Tables and Figures**

**Table S1.** Baseline characteristics of the patients

|  |  |  |  |
| --- | --- | --- | --- |
|  | | n=110 | |
| Men, n (%) | | 74 | (SD: 67.3) |
| Age (y), mean (SD) | | 40.3 | (SD: 12.5) |
| Biomarker | |  |  |
|  | LDH (U/L) | 264.8 | (SD: 73.4) |
|  | Eosinophils (/μＬ) | 586.5 | (SD: 506.9) |
|  | Total IgE (IU/mL) | 11091.9 | (SD: 9082.4) |
|  | SolubleIL-2R (U/mL) | 416.7 | (SD: 201.3) |
|  | TARC (pg/mL) | 2823.1 | (SD: 4306.4) |
|  | CCL22 (pg/mL) | 1424.9 | (SD: 1006.8) |
|  | CCL26 (pg/mL) | 43.1 | (SD: 39.4) |
|  | IL-13 (pg/mL) | 0.3 | (SD: 0.2) |
|  | IL-22 (pg/mL) | 8.5 | (SD: 15.6) |
|  | IL-31 (pg/mL) | 1.2 | (SD: 2.6) |
|  | CCL27 (pg/mL) | 1087.2 | (SD: 417.7) |
|  | CCL18 (pg/mL) | 127461.1 | (SD: 99681.4) |
|  | IL-24 (pg/mL) | 17.3 | (SD: 7.4) |
|  | IL-25 (pg/mL) | 128.4 | (SD: 25.5) |
|  | IL-33 (pg/mL) | 3.1 | (SD: 0.0) |
|  | TSLP (pg/mL) | 32.4 | (SD: 7.0) |
|  | ET1 | 1.1 | (SD: 0.4) |
|  | Periostin | 103.7 | (SD: 52.2) |
|  | SCCA2 | 8.3 | (SD: 14.7) |
| Outcome | |  |  |
|  | EASI score, mean (SD) | 26.6 | (SD: 9.9) |
|  | POEM score, mean (SD) | 18.2 | (SD: 6.9) |
|  | pruritus-NRS, mean (SD) | 6.5 | (SD: 2.3) |
|  | uncomfortable skin-NRS, mean (SD) | 6.3 | (SD: 2.7) |
|  | treatment satisfaction-NRS, mean (SD) | 5.1 | (SD: 2.7) |

**Table S2.** Association between baseline levels of biomarkers and % change from baseline of uncomfortable skin-NRS at 16 w of dupilumab treatment

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Biomarker | uncomfortable skin-NRS | | | |
| Coefficient | 95% CI | | P value |
| log(LDH) | -85.239 | (-222.857, | 52.378) | 0.222 |
| Eosinophils | -0.018 | (-0.048, | 0.012) | 0.228 |
| Total IgE | 0.000 | (-0.002, | 0.002) | 0.984 |
| SolubleIL-2R | -0.082 | (-0.157, | -0.008) | 0.030 |
| log(TARC) | -32.737 | (-64.190, | -1.274) | 0.042 |
| CCL22 | -0.010 | (-0.025, | 0.005) | 0.206 |
| CCL26 | -0.154 | (-0.536, | 0.229) | 0.427 |
| IL-13 | -67.791 | (-167.770, | 32.190) | 0.182 |
| IL-22 | -0.460 | (-1.422, | 0.501) | 0.344 |
| IL-31 | 1.977 | (-3.732, | 7.686) | 0.494 |
| CCL27 | -0.025 | (-0.063, | 0.012) | 0.183 |
| CCL18 | -0.0001 | (-0.0003, | 0.0000) | 0.111 |
| IL-24 | 0.196 | (-1.869, | 2.262) | 0.851 |
| IL-25 | 0.006 | (-0.583, | 0.596) | 0.983 |
| IL-33 | (omitted) |  |  |  |
| TSLP | -0.568 | (-2.732, | 1.596) | 0.604 |
| ET1 | -4.999 | (-39.432, | 29.435) | 0.774 |
| Periostin | -0.104 | (-0.399, | 0.190) | 0.484 |
| SCCA2 | -0.569 | (-1.590, | 0.453) | 0.272 |

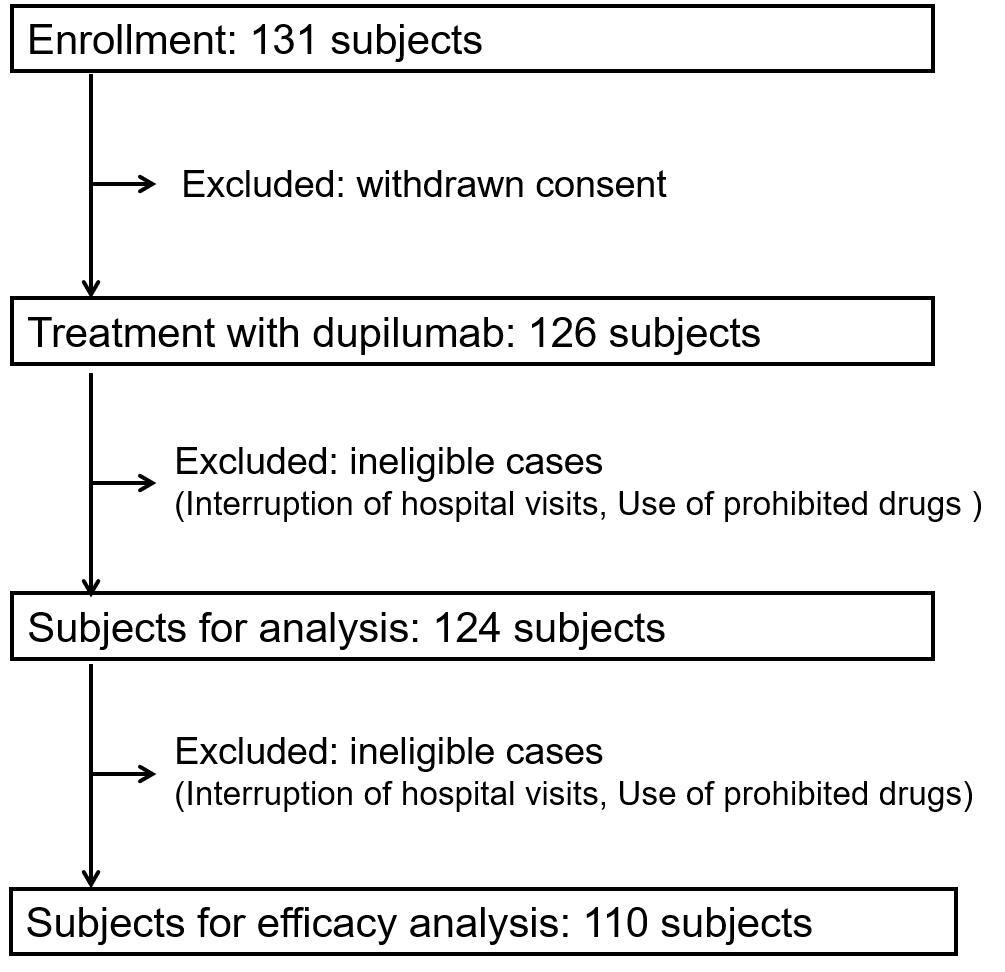
\*LDH and TARC were log-transformed.

**Table S3.** Association between baseline levels of biomarkers and % change from baseline of treatment satisfaction-NRS at 16 w of dupilumab treatment

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Biomarker | treatment satisfaction-NRS | | | |
| Coefficient | 95% CI | | P value |
| log(LDH) | -63.579 | (-374.098, | 246.941) | 0.685 |
| Eosinophils | 0.004 | (-0.079, | 0.088) | 0.915 |
| Total IgE | 0.001 | (-0.003, | 0.004) | 0.736 |
| SolubleIL-2R | 0.033 | (-0.140, | 0.207) | 0.706 |
| log(TARC) | 48.287 | (-22.021, | 118.595) | 0.176 |
| CCL22 | 0.012 | (-0.024, | 0.047) | 0.515 |
| CCL26 | -0.141 | (-1.011, | 0.729) | 0.748 |
| IL-13 | 229.744 | (-18.286, | 477.774) | 0.069 |
| IL-22 | 0.422 | (-1.698, | 2.542) | 0.694 |
| IL-31 | -3.718 | (-16.259, | 8.822) | 0.558 |
| CCL27 | 0.010 | (-0.077, | 0.097) | 0.822 |
| CCL18 | 0.0000 | (-0.0003, | 0.0004) | 0.858 |
| IL-24 | -2.015 | (-6.508, | 2.477) | 0.375 |
| IL-25 | -0.404 | (-1.696, | 0.887) | 0.536 |
| IL-33 | (omitted) |  |  |  |
| TSLP | -2.103 | (-6.841, | 2.635) | 0.380 |
| ET1 | -24.601 | (-101.050, | 51.849) | 0.524 |
| Periostin | 0.035 | (-0.671, | 0.741) | 0.922 |
| SCCA2 | -0.017 | (-2.306, | 2.271) | 0.988 |

\*LDH and TARC were log-transformed.

**Figure S1.** Flow sheet of the study and investigations performed



**Figure S2.**

**Pruritus-NRS**

Please indicate the intensity of peak pruritus, or worst itch, over the previous 24 hours.

0 1 2 3 4 5 6 7 8 9 10

No itch Worst itch imaginable

**Uncomfortable skin-NRS**

Please grade “uncomfortableness” of your skin

0 1 2 3 4 5 6 7 8 9 10

No uncomfortableness Worst uncomfortableness imaginable

**Treatment satisfaction-NRS**

Please grade “satisfaction” of the treatment

0 1 2 3 4 5 6 7 8 9 10

Unsatisfied at all Satisfied very much

**Figure S3.** Study calendar

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Week | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
| Day | Day0 | Day14 | Day28 | Day42 | Day56 | Day70 | Day84 | Day98 | Day112 |
| Injection of dupilumab | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Patient characteristics | ○ |  |  |  |  |  |  |  |  |
| EASI | ○ | ○ | ○ |  | ○ |  |  |  | ○ |
| POEM | ○ | ○ | ○ |  | ○ |  |  |  | ○ |
| pruritus-NRS | ○ | ○ | ○ |  | ○ |  |  |  | ○ |
| uncomfortable skin-NRS | ○ | ○ | ○ |  | ○ |  |  |  | ○ |
| treatment-satisfaction NRS | ○ | ○ | ○ |  | ○ |  |  |  | ○ |
| Blood Sampling (Biomarkers) | ○ |  |  |  |  |  |  |  |  |