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Concise Report

Methotrexate and ciclosporin both reduce levels of circulating interleukin (IL)-4 and IL-13 expressing CD4+ memory T cells in childhood atopic dermatitis

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Abstract

Atopic dermatitis (AD) is a chronic dermatosis characterized by type-2 inflammatory responses, skin barrier anomalies and microbiome dysregulation. The variety of AD presentations necessitates a better understanding of the underlying disease mechanisms and the modulation of immune markers over a treatment course. Globally, the most used systemic therapies for moderate-to-severe AD are methotrexate (MTX) and ciclosporin (CyA). The TReatment of severe Atopic Eczema in children Trial (TREAT) was a randomized controlled trial (RCT) assessing the efficacy and safety of MTX and CyA. Peripheral blood samples from 18 TREAT participants were analysed in a longitudinal immunological study with a focus on cytokine-expressing CD4+ T cells. The analysis showed that both MTX and CyA were associated with a decreased percentage of interleukin (IL)-4 and IL-13 expressing CD4+ memory T cells, corresponding to improved disease severity. Patients receiving MTX experienced a more sustained decrease in IL-4 expressing T cells, which corresponds to the longer-term improved disease control observed in the MTX arm.

Atopic dermatitis (AD) (also called 'eczema') is a prevalent inflammatory dermatosis, characterized by a type 2 inflammatory response, inherited and acquired barrier anomalies and microbiome dysregulation.[1] AD ranks 15th among all nonfatal diseases and first among all skin diseases with large phenotypical and geographical variation, making AD a significant global health issue.[2] The global prevalence of AD imposes a substantial burden on healthcare systems, affecting paediatric populations in particular.[2] This chronic condition not only impacts physical health but also significantly affects the quality of life for patients and their families.[3,4]

The multifaceted nature of AD necessitates a comprehensive understanding of its underlying mechanisms and the use of effective therapeutic interventions to improve symptoms and patient outcomes. Currently, methotrexate (MTX) and ciclosporin (CyA) serve as the main conventional systemic treatments for severe AD globally. Despite the emergence of novel therapies, there remains a significant gap in understanding the complex interaction of immune biomarkers and their modulation throughout the disease course of AD. We recently conducted the Treatment of severe Atopic Eczema in children Trial (TREAT), a multicentre, parallel group, assessor-blinded superiority RCT (EudraCT 2015-002013-29).[5] The trial confirmed that CyA and MTX are effective treatments for severe AD in paediatric patients.[6] CyA led to faster disease control, while MTX showed sustained disease control post-therapy. We present a longitudinal immunological analysis of peripheral blood samples collected from 18 participants (CyA, $n=11$; MTX, $n=7$) in the TREAT trial at baseline and during follow-up, with a focus on cytokine-producing CD4+ T cells in the two treatment arms.

Report

TREAT was conducted at 13 paediatric dermatology departments across the UK and Ireland. Eligible patients were between 2 and 16 years old, had severe recalcitrant atopic eczema defined as an objective Severity Scoring of Atopic Dermatitis (O-SCORAD) index ≥ 30 and an inadequate response to potent topical treatment. A full list of inclusion

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and exclusion criteria can be found in the protocol. Once all baseline assessments had been performed, participants were randomized to a study drug in a 1 : 1 ratio, which was then dispensed by the local hospital pharmacy. All participants were seen at weeks 2, 4, 8, 12, 20, 28, 36, 48 and 60 for efficacy and safety parameters.

Blinding of the local investigator, research nurse and participants was not possible because of the nature of the trial interventions. The assessor performing the severity assessments was blinded to the treatment group and all laboratory personnel were blinded to treatment allocations until the end of the trial.

Participants randomized to the CyA arm (Neoral, Novartis Pharmaceuticals, London, UK) were prescribed 4 mg kg^{-1} daily orally in two divided doses for the treatment period of 36 weeks. After 12 weeks, dose increases (to a maximum of 5 mg kg^{-1} daily) or decreases were allowed, dependent on individual treatment response. Participants randomized to the MTX arm (any brands with UK/European Union marketing authorization) were prescribed a single test dose of 0.1 mg kg^{-1} at week 0 and then 0.4 mg kg^{-1} weekly orally (maximum dose 25 mg per week) until week 36. Only MTX 2.5 mg strength tablets were dispensed. Participants in the MTX arm were also prescribed oral folic acid 1 mg once daily apart from on the day of MTX administration. Both MTX and CyA proved effective to treat severe AD in paediatric patients. CyA displayed a more rapid onset of results, while MTX provided more sustained disease control.[6]

For the immunological study, peripheral blood mononuclear cells (PBMCs) were collected and cryopreserved at baseline and at 12, 36 (when treatment was stopped) and 60 weeks. For our longitudinal flow cytometry analysis, we included those participants for whom either all four or at least three of four timepoints were available, always including baseline samples. For a proportion of the participants, certain timepoints were not available because there were too few PBMCs, cells did not pass viability quality control upon cryopreservation, or too few high-quality events were acquired on the flow cytometer.

Upon thawing, 2×10^6 PBMCs were cultured with 50 ng mL^{-1} phorbol myristate acetate and 750 ng mL^{-1} ionomycin in the presence of GolgiStop (BD Biosciences, Franklin Lakes, NJ, USA) for 3 h, followed by staining for viability and the indicated extracellular and intracellular molecules using saponin. To account for variation in age between participants (6–15 years), we gated on $CD45RO^+$ $CD45RA^-$ memory $CD4^+$ T cells to normalize for possible age-related differences in the proportion of memory T cells (Figure 1). The analysis followed the intention-to-treat principle, with participants analysed in the group to which they were randomized. Results are presented using individual profile plots and box plots over time for 18 participants who provided analysable samples. Responders were defined as those reaching at least a 50% reduction in Eczema Area and Severity Index (EASI) disease severity score from baseline by week 36 (EASI-50).

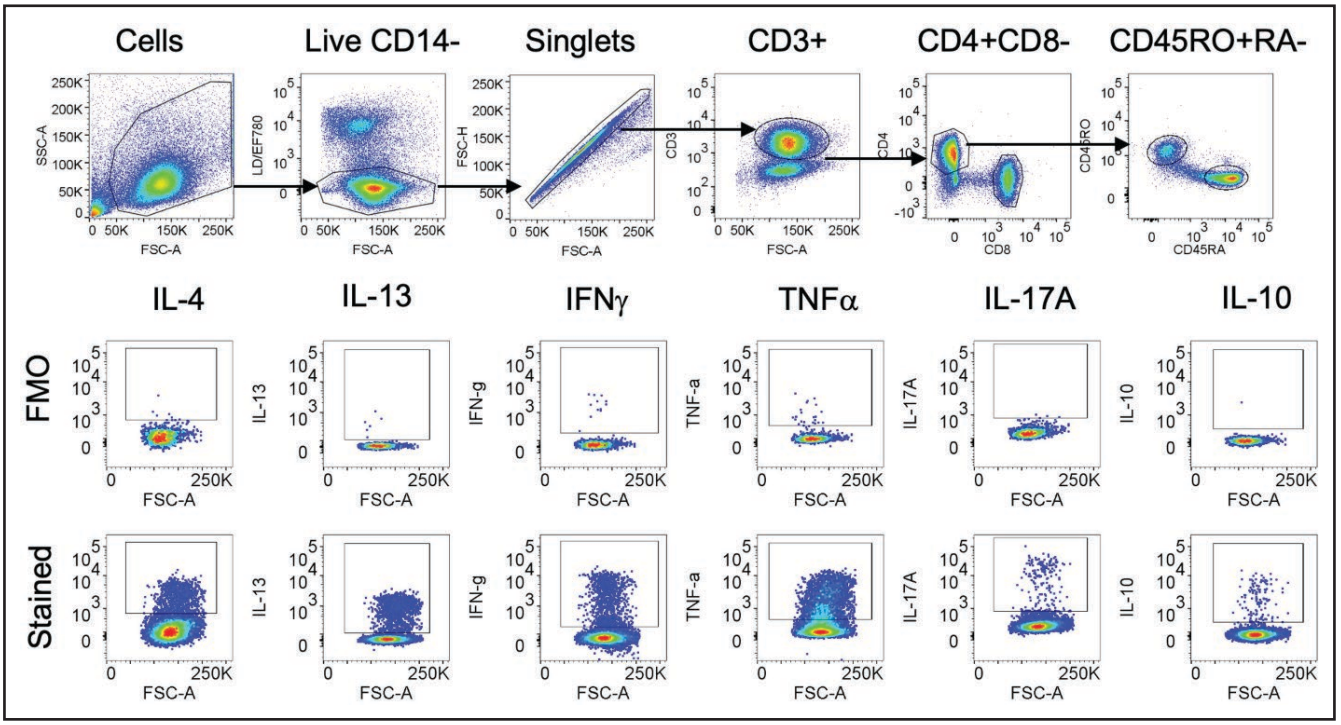

Figure 1 Gating strategy for identification and analysis of cytokine-expressing $CD4^+$ T cells in peripheral blood from TREAT participants. Peripheral blood mononuclear cells (PBMCs) were thawed and stimulated for 3 h with phorbol myristate acetate and ionomycin in the presence of GolgiStop, followed by staining for the presence of the indicated molecules. (Top row) Gating strategy to identify memory $CD4^+$ T cells in PBMCs using consecutive gating for mononuclear cells, live $CD14^-$ cells, single cells, $CD3^+$ cells, $CD4^+CD8^-$ cells and $CD45RO^+CD45RA^-$ cells. (Middle and bottom rows) Representative staining showing cells expressing the indicated cytokines within $CD3^+CD4^+CD45RO^+$ T cells of one TREAT participant. Fluorescence minus one controls were used to set gates for analysis. FMO, fluorescence minus one; FSC-A, forward scatter area; FSC-H, forward scatter height; IFN, interferon; IL, interleukin; SSC-A, side scatter area; TNF, tumour necrosis factor.

When all TREAT participants were analysed together, we observed a decreasing trend in the percentage of interleukin (IL)-4 and IL-13 expressing CD4+ memory T cells over time (Figure 2). When responder vs. nonresponder status was defined at week 36, the decreases in IL-4 and IL-13 expressing CD4+ T cells appeared more striking in the responder group compared with the nonresponder group (Figure 2). The decrease appeared more visible for patients treated with MTX than for those in the ciclosporin group and was more sustained for IL-4 expressing T cells up to week 60, even though treatment was ceased at 36 weeks.

The percentages of interferon-γ (type-1) and IL-17A (type-17) expressing CD4+ memory T cells did not show particular trends over time, while the percentage of tumour necrosis factor-α expressing memory CD4+ T cells appeared to decrease over time up to week 60 in all patients, irrespective of response or treatment (Figure 3). These data suggest that the decrease was specific to CD4+ T cells producing type 2 cytokines. The percentage of anti-inflammatory IL-10 expressing memory CD4+ T cells decreased initially, with a reversal to baseline levels at week 36 when treatment was stopped. This was particularly noticeable in patients treated with MTX.

Our study is limited by the small number of patient samples at each timepoint, particularly when considering the different treatment and response groups. Nonetheless, the analysis indicates that both CyA and MTX treatments

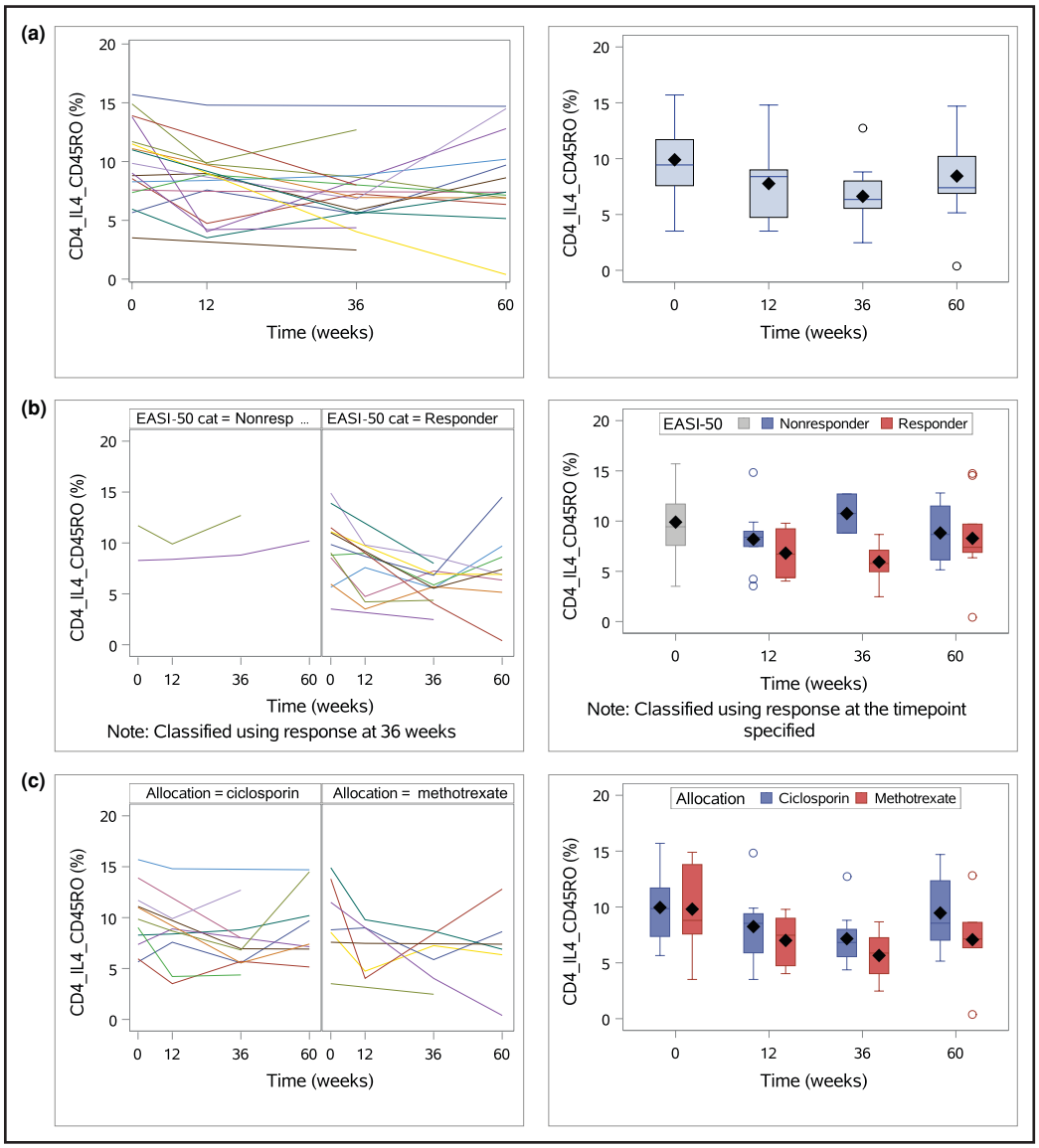

Figure 2 Interleukin (IL)-expressing memory CD4+ T cells in TREAT participants over time. (a–c) IL-4. (d–f) IL-13. Peripheral blood mononuclear cells were stimulated with phorbol myristate acetate/ionomycin in the presence of GolgiStop and cytokine-expressing memory CD4+ T cells were identified. Individual profile plots and box plots of percentages of (a) IL-4+ or (d) IL-13+ memory CD4+ T cells are shown over time (baseline *n*=18; week 12 *n*=13; week 36 *n*=14; week 60 *n*=14), (b, e) split by treatment response defined as reaching EASI-50 (baseline *n*=18; week 12 responder *n*=4, nonresponder *n*=9; week 36 responder *n*=12, nonresponder *n*=2; week 60 responder *n*=10, nonresponder *n*=4) or (c, f) by treatment group [baseline ciclosporin (CyA) *n*=11, methotrexate (MTX) *n*=7; week 12 CyA, *n*=8 MTX *n*=5; week 36 CyA *n*=9, MTX *n*=5; week 60 CyA *n*=8, MTX *n*=6). EASI, Eczema Area and Severity Index. (*Continued*)

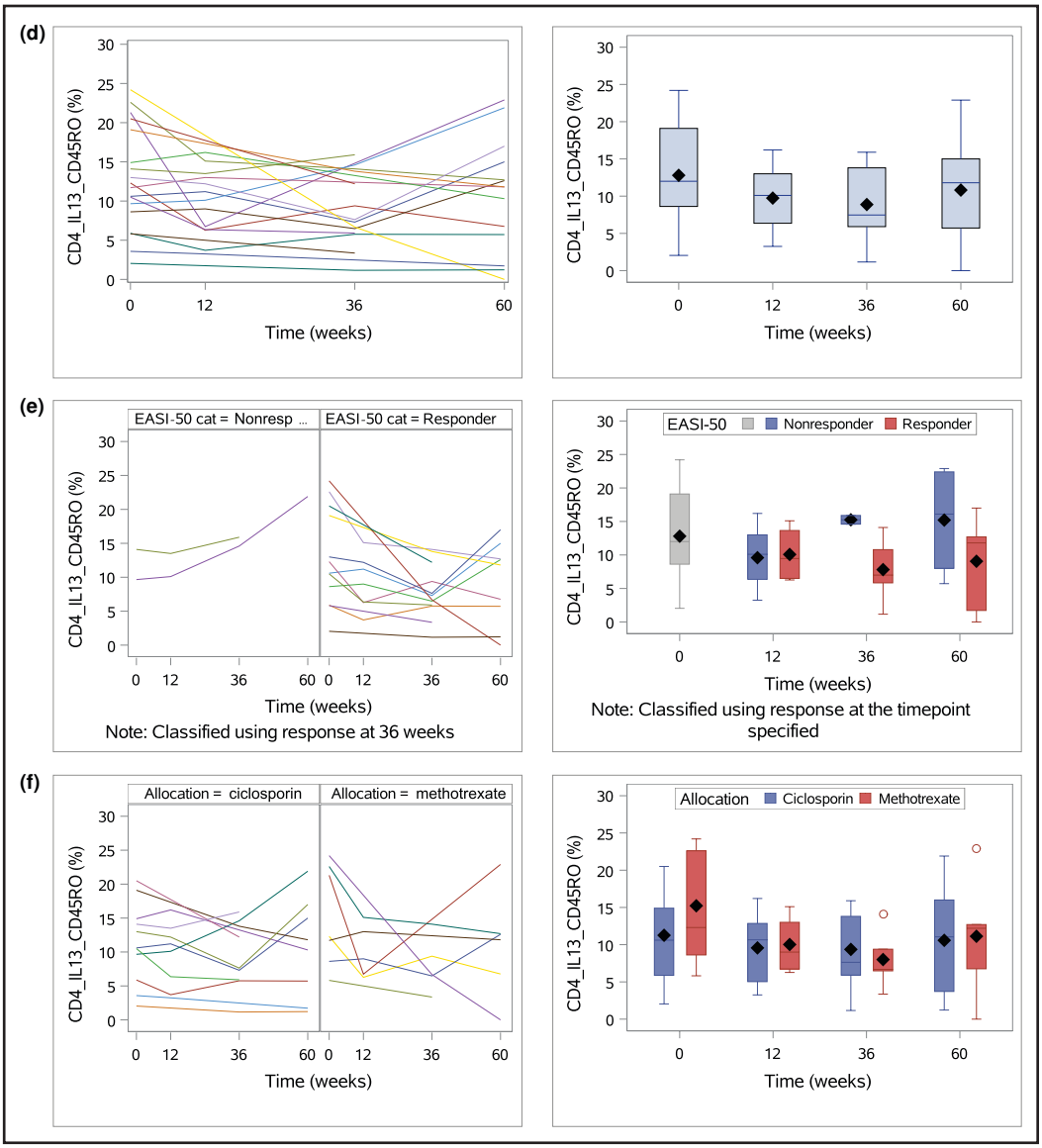

Figure 2 (*Continued*)

are associated with a decrease in the percentage of IL-4 and IL-13 expressing CD4+ memory T cells, corresponding with improvement in disease severity. Our data support Bunikowski *et al.*'s previous findings that treatment with CyA led to a significant decrease in the frequency of IL-4- and IL-13-producing CD3+ T cells in paediatric patients with AD,[7] while Roekevisch *et al.* found no significant reduction in serum IL-13 levels in an adult population with AD.[8] The decrease in IL-4 expressing T cells may be sustained longer in those who received MTX and reflects the improved overall disease control we observed during the 6 months off therapy in this group of patients, compared with those who had received CyA.[6] The results from this study serve to bolster the knowledge base on the underlying disease mechanisms of AD and the ways in which MTX and CyA modulate immune markers.

Learning points

- Methotrexate and ciclosporin treatment are both associated with a decreased percentages of interleukin (IL)-4 and IL-13 expressing CD4+ memory T cells.
- Patients in the methotrexate treatment arm exhibited a more sustained decrease in IL-4 expressing T cells compared with the ciclosporin arm.
- The decrease in cytokine-expressing CD4+ T cells stopped once treatment ended in week 36.

Funding sources

The UK Medical Research Council/National Institute for Health Research Efficacy and Mechanism Evaluation Board provided the financial resources for the conduct of the trial

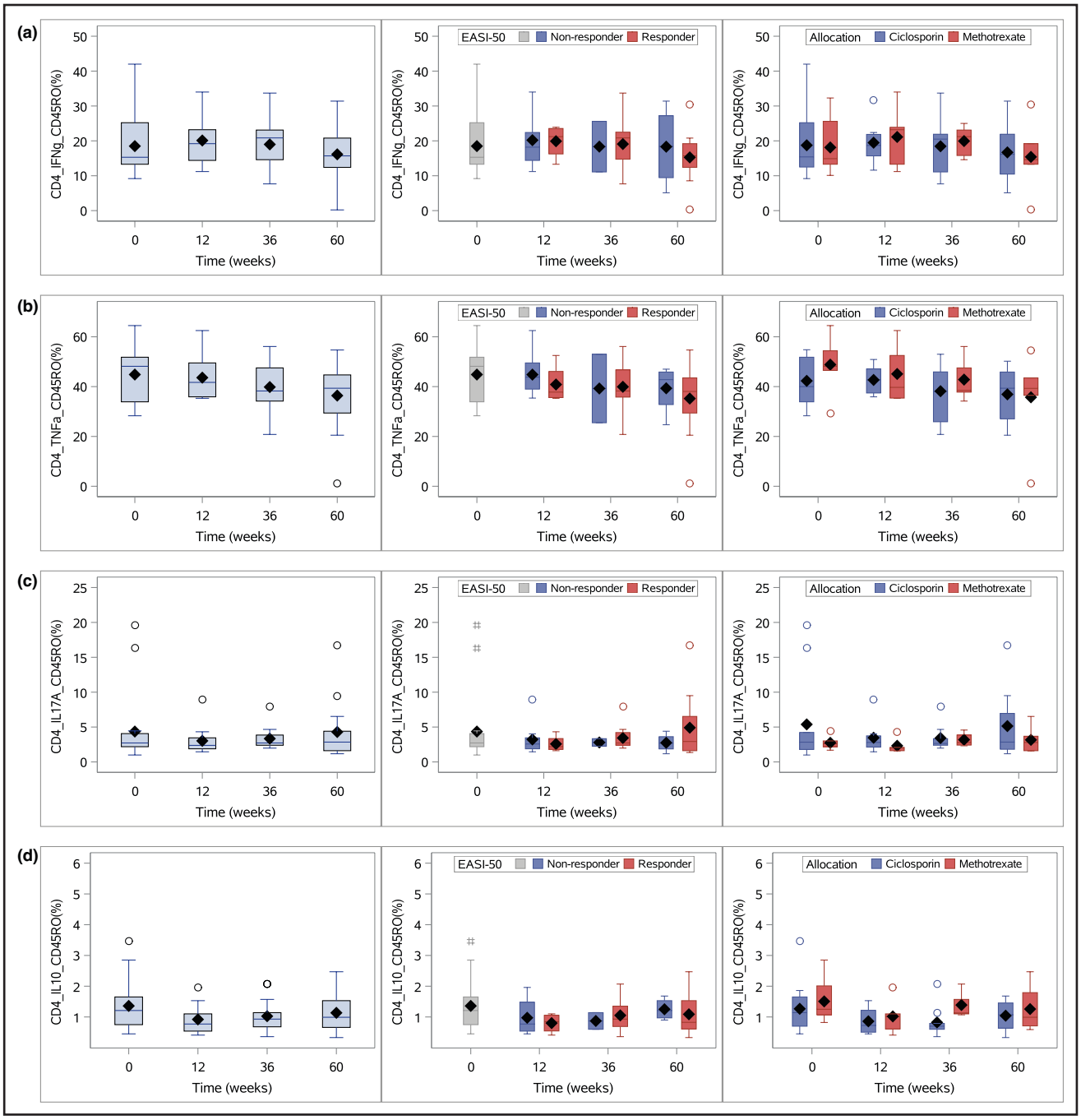

Figure 3 Percentage of additional cytokine-expressing cells within the CD4⁺ T cell memory population over time. Peripheral blood mononuclear cells were stimulated with phorbol myristate acetate/ionomycin in the presence of GolgiStop followed by intracellular cytokine staining. Memory CD4⁺ T cells were gated and percentages of cells expressing the indicated cytokines were identified. Box plots of percentages of (a) interferon-γ⁺, (b) tumour necrosis factor-α⁺, (c) interleukin (IL)-17A⁺ and (d) IL-10⁺ memory CD4⁺ T cells are shown over time (left column), baseline $n=18$; week 12 $n=13$; week 36 $n=14$; week 60 $n=14$, split by treatment response defined as reaching EASI-50 (middle column), baseline $n=18$, week 12 responder $n=4$, nonresponder $n=9$; week 36 responder $n=12$; nonresponder $n=2$; week 60 responder $n=10$, nonresponder $n=4$, or by treatment group (right column), baseline ciclosporin (CyA) $n=11$, methotrexate (MTX) $n=7$; week 12 CyA $n=8$, MTX $n=5$; week 36 CyA $n=9$, MTX $n=5$; week 60 CyA $n=8$, MTX $n=6$). EASI, Eczema Area and Severity Index.

(grant code 15/EE/0328), provided continuing support to the chief investigator to ensure that the trial progressed smoothly and monitored progress against key milestones via the submission of regular progress reports. G.S.O. is funded by the Medical Research Council UK. The funders had no influence on the participant enrolment and follow-up, data collection, data analyses and writing of this manuscript.

Conflicts of interest

C.F. is Chief Investigator of the UK National Institute for Health Research-funded TREAT (ISRCTN15837754) and SOFTER (Clinicaltrials.gov: NCT03270566) trials, as well as the UK–Irish Atopic eczema Systemic Therapy Register (ISRCTN11210918) and a principal investigator in the European Union (EU) Horizon 2020-funded BIOMAP

Consortium (www.biomap-imi.eu). He also leads the EU Trans-Foods consortium. His department has received investigator-led funding from Sanofi-Genzyme and Pfizer for microbiome work. A.D.I. has received consulting fees from Area, Almirall, Abbvie, Pfizer, Eli Lilly and Sanofi-Regeneron and is the President of the International Eczema Council. L.S.T. has received consultancy fees and/or research support from AbbVie, GSK, Sanofi and UCB outside of the reported work. G.S.O. has had relevant research collaborations with Johnson & Johnson and UCB and is co-inventor on relevant patents all administered through the employer. The other authors declare no conflicts of interest.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Ethics statement

The study protocol was approved by the Cambridge Central Research Ethics Committee (REC reference 15/EE/0328). The trial registration number is ISRCTN15837754 (registered 9 March 2016).

Patient consent

Not applicable.

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