# Treatment with Abrocitinib reduces circulating, skin homing, and resident memory T-cells in atopic dermatitis samples *in vitro* and *ex vivo*, and the severity of disease flares in a humanized mouse model

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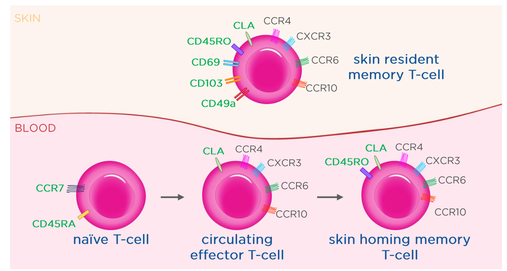
## Background

Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by immune cell activation, impairment of the skin barrier and waves of disease recurrence [1, 2]. AD recurrences are suggested to be mediated by memory T-cells (see Figure on the right) [3]. Key disease pathways of AD include the Th2-derived cytokines IL-13 and IL-4. Indeed, their blockade by biologics prompted significant improvements in patients with moderate-to-severe AD [4]. However, unlike other skin inflammatory diseases, targeting those cytokines only resolves AD in around 38% of the patients [5]. Thus, more efficient treatments are sought after, which also reduce disease reappearances. The janus activated kinase 1 (JAK1) inhibitor Abrocitinib has shown promising potential in clinical studies in AD patients [6]. JAK signalling is induced by various cytokines, including IL-4, -13, -31, -6, -15, -22 or interferons, and regulates T-cell differentiation and function [7]. Given its broad spectrum of activity, the beneficial effects of Abrocitinib in ameliorating AD may be superior to other therapeutics [6].




Naïve T-cells are characterized by CCR7 and CD45RA expression, which are downreguated on effector and memory T-cells. While blood circulating memory T-cells upregulate only CD45RO and CLA, skin resident memory T-cells are also positve for CD103, CD69 and CD49a. CCR4, CXCR3, CCR6, and CCR10 further distinguish Th1, Th2, Th17, Th17+1, and Th22 cells. Adapted from [8-11].

## Aim of the study

Here, we aimed at further dissecting the mechanism of Abrocitinib in ameliorating AD, focusing on its effect on circulating, skin homing, and skin resident memory T-cells.

## Take-home Message

"The AD disease relapse-preventing effects of abrocitinib are, at least in part, linked to its properties to interfere with the expansion and/or survival of memory T-cells"

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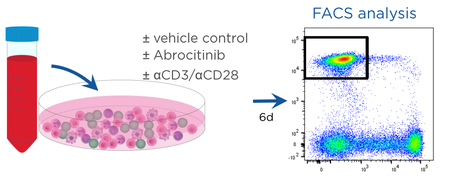
## Methods

*In vitro* - Analysis of AD PBMCs




Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of 8 atopic dermatitis (AD) patients and treated with vehicle control (0.1% DMSO) or 5µM Abrocitinib in the presence or absence of T-cell receptor stimulation with 0.5µg/ml αCD3/αCD28 antibodies. After 6 days of culture, cells were analysed by fluorescent activated cell sorting (FACS).

## Results

### Abrocitinib significantly reduces the number of CD4⁺/CD8⁺CD45RO⁺ memory T-cells and CD8⁺CD45RO⁺CLA⁺ skin homing memory T-cells in αCD3/αCD28-stimulated AD PBMCs

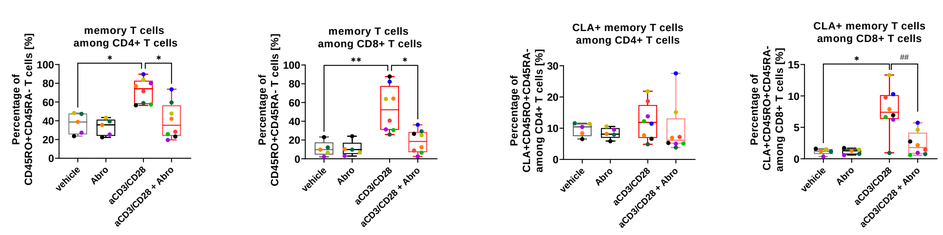



Figure 1. The percentage of (a) CD4⁺/CD8⁺CD45RO⁺ memory T-cells and (b) CD8⁺CD45RO⁺CLA⁺ skin homing memory T-cells among CD3⁺ cells in αCD3/αCD28-stimulated AD PBMCs in the presence or absence of vehicle control or Abrocitinib was assessed by FACS, gated on CD3⁺ cells. n=5-8 AD donors. Mann-Whitney test ##p<0.01. Kruskal-Wallis-test *p<0.05, **p<0.01. Dots of different colour represent different AD patients.

### Abrocitinib significantly reduces Tc1 memory T-cells among αCD3/αCD28-stimulated CD45RO⁺CD8⁺T-cells

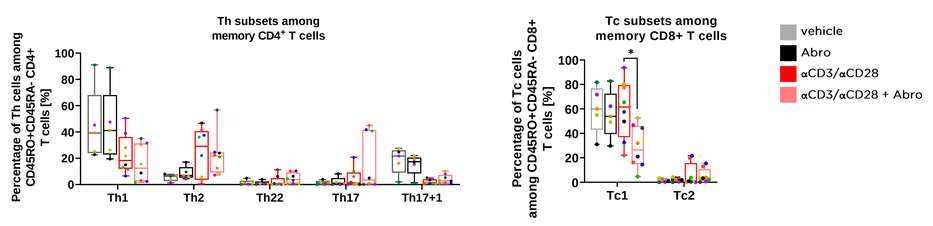
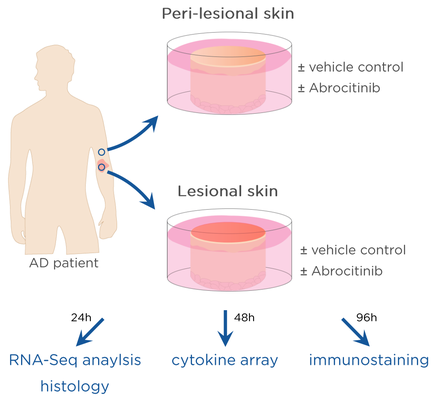



Figure 2. The percentage of different helper T-cell (Th) subtypes among CD4⁺CD45RO⁺ memory T-cells (a) and the percentage of different cytotoxic T-cells (Tc) among CD8⁺CD45RO⁺ memory T-cells (b) in αCD3/αCD28-stimulated AD PBMCs in the presence or absence of vehicle control or Abrocitinib was assessed by FACS, gated on CD3⁺ cells n=5-8 AD donors. Data were obtained by FACS; GraphPad Prism 9, Kruskal-Wallis-test *p<0.05. Dots of different colour represent different AD patients.

*Ex vivo* – Analysis of lesional AD skin




Lesional and peri-lesional skin was isolated from 6-9 atopic dermatitis (AD) patients. The skin punches were cultured in the presence or absence of vehicle control (0.1% DMSO) or 5µM Abrocitinib. RNA-Seq analysis was performed after 24h, protein expression analyses after 96h, and cytokine arrays after 48h of *ex vivo* culture.

### Abrocitinib ameliorates the lesional transcriptomic phenotype in *ex vivo* cultured AD skin

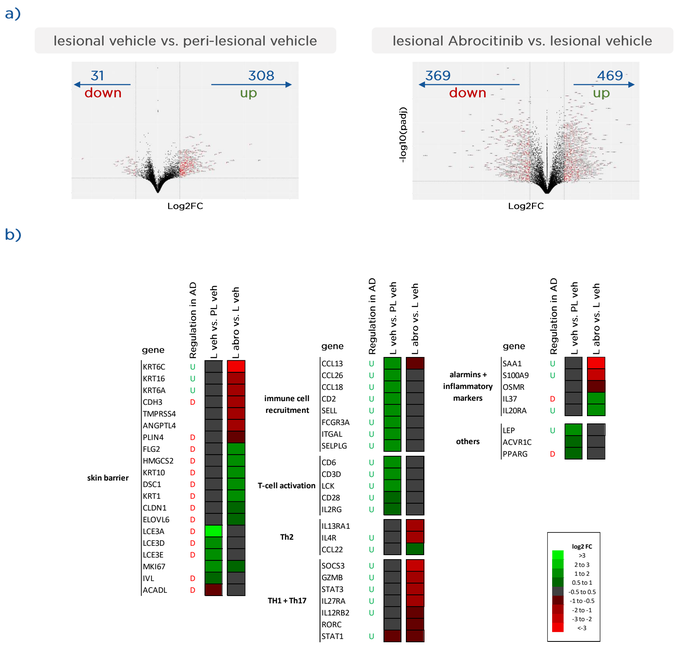



Figure 3. RNA-Seq analysis shows an increased inflammatory phenotype in vehicle treated lesional compared to vehicle treated peri-lesional atopic dermatitis (AD) skin, which is ameliorated by Abrocitinib treatment. (a) Volcano plot showing the significantly down regulated genes (DESeq2: padj ≤0.05, log2FC ≥1/≤-1) in lesional vehicle vs peri-lesional vehicle treated AD skin (left) and lesional Abrocitinib vs. lesional vehicle treated AD skin (right). (b) Heatmaps showing Log2 fold-change of differentially regulated genes in lesional (L) vehicle vs peri-lesional (PL) vehicle treated AD skin (left columns) in comparison to lesional Abrocitinib (abro) vs. lesional vehicle treated AD skin (right columns). The left columns show the disease regulation, e.g. active immune responses in lesional AD skin, and the right columns show the treatment effect of Abrocitinib. n = 6 donors were analysed. Displayed genes have been described to be dysregulated in AD (D: down-regulated in AD, U: up-regulated in AD).

### Abrocitinib tendentially decreases cytokine secretion in peri-lesional and/or lesional AD skin

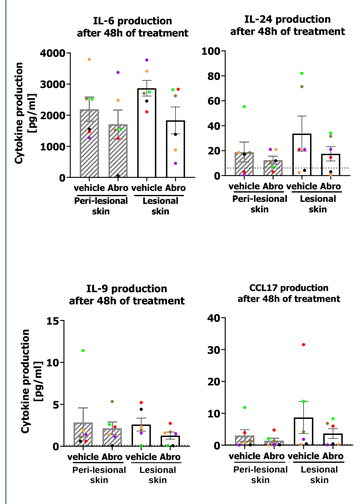



Figure 4. Analysis of the supernatant from lesional and peri-lesional atopic dermatitis (AD) skin in the presence or absence of vehicle control or abrocitinib for 48h *ex vivo*. Interleukin-6 (IL-6), IL-24, IL-9 and CCL17/TARC (CC chemokine ligand 17/thymus and activation-regulated chemokine) secretion were assessed by EVE technologies cytokine array. n=6 patients. mean±SEM; D'Agostino & Pearson test for normal distribution, Kruskal-Wallis test and Dunn´s multiple comparison n.s; Mann-Whitney-Test n.s. Dots of different colour represent different AD patients.

### Abrocitinib significantly reduces the number of resident CD45RO⁺CD69⁺ memory T-cells in the epidermis of peri-lesional AD skin, and that of CD45RO⁺CLA⁺ skin homing memory T-cells in the epidermis of lesional AD skin

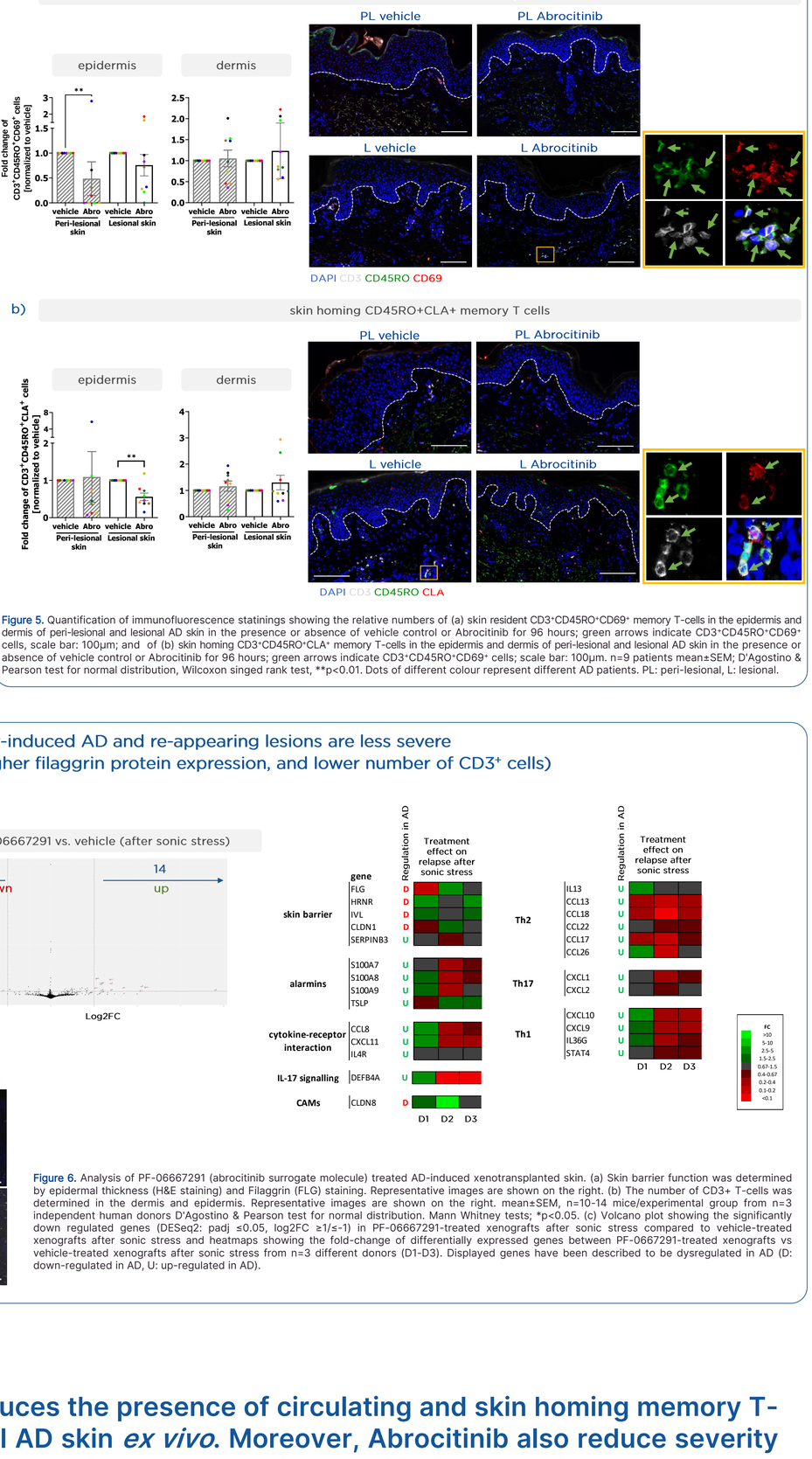
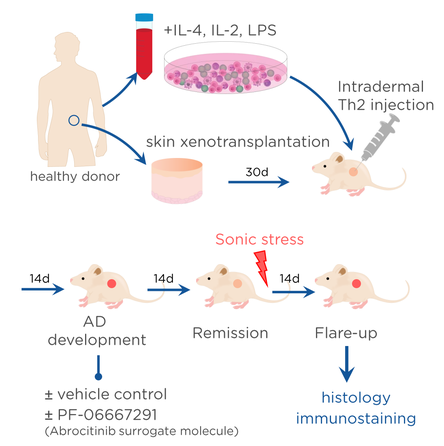



Figure 5. Quantification of immunofluorescence statinings showing the relative numbers of (a) skin resident CD3⁺CD45RO⁺CD69⁺ memory T-cells in the epidermis and dermis of peri-lesional and lesional AD skin in the presence or absence of vehicle control or Abrocitinib for 96 hours; green arrows indicate CD3⁺CD45RO⁺CD69⁺ cells, scale bar: 100µm; and of (b) skin homing CD3⁺CD45RO⁺CLA⁺ memory T-cells in the epidermis and dermis of peri-lesional and lesional AD skin in the presence or absence of vehicle control or Abrocitinib for 96 hours; green arrows indicate CD3⁺CD45RO⁺CD69⁺ cells; scale bar: 100µm. n=9 patients mean±SEM; D'Agostino & Pearson test for normal distribution, Wilcoxon singed rank test, **p<0.01. Dots of different colour represent different AD patients. PL: peri-lesional, L: lesional.

*In vivo* – Humanized mouse model of AD [12]




Abdominal skin from 3 healthy donors was transplanted onto 60 beige-SCID mice. 30 days after transplantation, the grafted skin was injected intradermally with autologous *in vitro* IL-4,IL-2,LPS-treated PMBCs to induce AD. 14 days after PBMC injection, treatments started. Mice received a daily oral administration of 2% Tween 20/0.5% methylcellulose (drug-vehicle) or PF-06667291 (abrocitinib surrogate molecule) for another 14 days. One day after treatment was discontinued, mice were exposed to sonic stress for 24h. 14 days later, the experiment was terminated and xenografts were used for further analysis

### Treatment with PF-06667291 significantly ameliorates experimentally-induced AD and re-appearing lesions are less severe (transcriptome revealing stronger skin barrier and lower overall inflammation, higher filaggrin protein expression, and lower number of CD3⁺ cells)

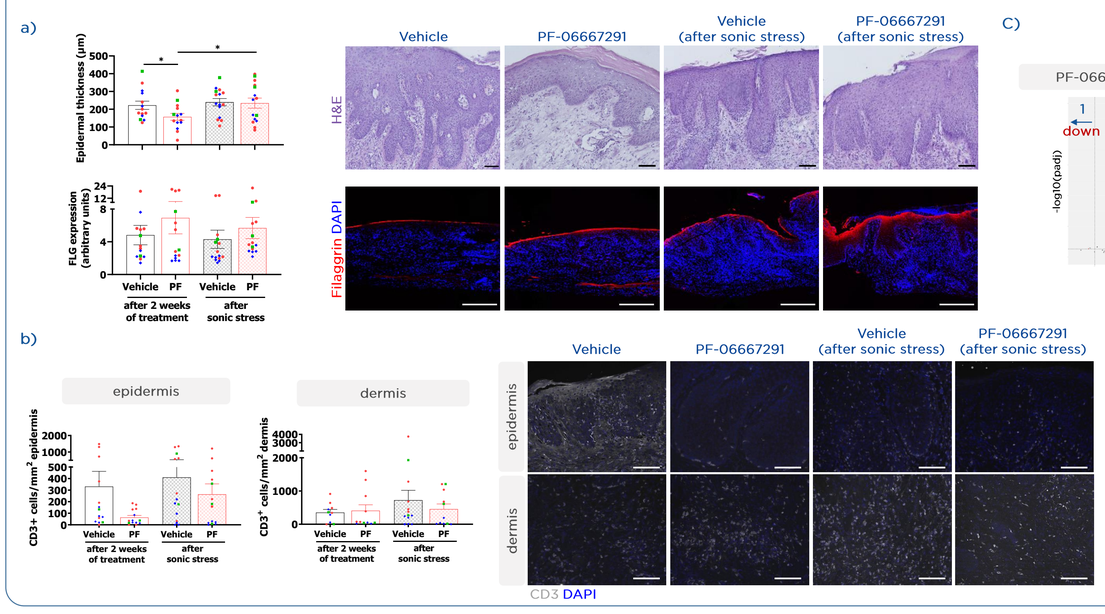



Figure 6. Analysis of PF-06667291 (abrocitinib surrogate molecule) treated AD-induced xenotransplanted skin. (a) Skin barrier function was determined by epidermal thickness (H&E staining) and Filaggrin (FLG) staining. Representative images are shown on the right. (b) The number of CD3+ T-cells was determined in the dermis and epidermis. Representative images are shown on the right. mean±SEM, n=10-14 mice/experimental group from n=3 independent human donors D'Agostino & Pearson test for normal distribution. Mann Whitney tests; *p<0.05. (c) Volcano plot showing the significantly down regulated genes (DESeq2: padj ≤0.05, log2FC ≥1/≤-1) in PF-06667291-treated xenografts after sonic stress compared to vehicle-treated xenografts after sonic stress and heatmaps showing the fold-change of differentially expressed genes between PF-0667291-treated xenografts vs vehicle-treated xenografts after sonic stress from n=3 different donors (D1-D3). Displayed genes have been described to be dysregulated in AD (D: down-regulated in AD, U: up-regulated in AD).

## Conclusion

Taken together, our preliminary data show that Abrocitinib treatment reduces the presence of circulating and skin homing memory T-cells *in vitro*, and of skin homing and -resident memory T-cells in lesional AD skin *ex vivo*. Moreover, Abrocitinib also reduce severity of experimentally-induced AD flare up in human xenotransplants. Thus, our findings suggest that the AD disease relapse-preventing effects of Abrocitinib result, at least in part, from its property to interfere with the expansion and/or survival of memory T-cells.

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DISCLOSURES

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