# Characterization of a model of alopecia areata in C3H/HeJ mice induced by adoptive transfer of lymph node cells

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Introduction

- Alopecia areata is a cell-mediated autoimmune disorder characterized by transient, non-scarring hair loss and preservation of the hair follicle. Skin biopsies of alopecia areata affected skin show a lymphocytic infiltrate in and around the bulb or the lower part of the hair follicle in anagen (hair growth) phase. A breakdown of immune privilege of the hair follicle is thought to be an important driver of alopecia areata.

- The C3H/HeJ strain of mice spontaneously develops alopecia and has been commonly used in animal models of the disease.

- In the present study, lymph node cells of spontaneous alopecic C3H/HeJ mice are obtained, expanded, and inoculated to healthy animals to increase the number of alopecic mice available.

- The model has been further characterized assessing the expression of different genes compared to human AA patients, observing in both cases an increase of disease related markers such as Cd8, Cxcl9, Cxcl10 and Klrk1. In addition, in alopecic animals increase of CD8+NKG2D+ cells in lymph nodes is observed by flow cytometry as well as CD8+ cells infiltration in skin by immunohistochemistry.

- JAK inhibitors have been shown to be effective in AA patients, therefore, in the pharmacological characterization of the model, the effect of ruxolitinib and baricitinib was compared in terms of hair recovery and gene expression profile in the skin. Both treatments reduced the alopecia score and CD8+ cells infiltration in the skin as well as expression of Cd8, Cxcl9, Cxcl10, Cxcl11, Klrk1 and Ifng.

- Overall, our results show that this model replicates several AA features and respond to oral JAK inhibitors, making it suitable for evaluating novel drugs for AA.
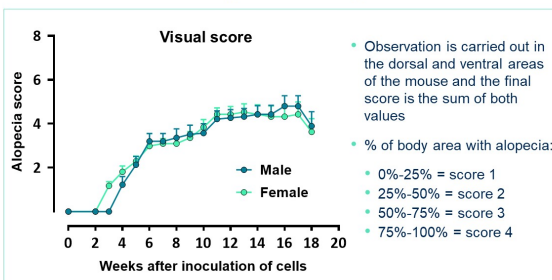
Material and Methods
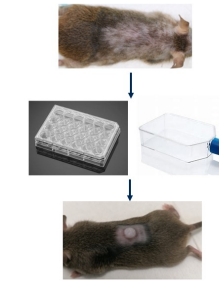
- Lymph node cells are obtained from mice with spontaneous alopecia

- Cells are expanded in AR10 medium (RPMI 1640 with 10% FBS, 2mM GlutaMAX and 100 U/mL Penicillin-Streptomycin) + IL7 + IL15 + IL2 + CD3/CD28 Dynabeads during 6 days

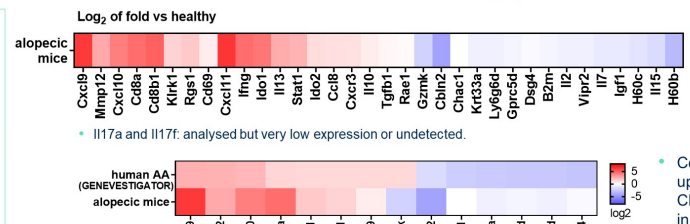- Cells are intradermically inoculated in animals without alopecia (10×10^6 cells/animal)

All *in vivo* experiments were approved and monitored by the Animal Ethical committee of Almirall following ARRIVE guidelines and in accordance with EU Directive 2010/63/EU.

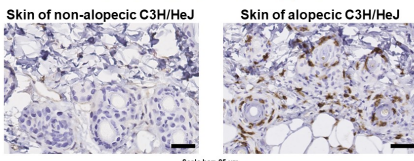## Model characterization

Alopecia development

Visual score

- Observation is carried out in the dorsal and ventral areas of the mouse and the final score is the sum of both values

- % of body area with alopecia:
  - 0%-25% = score 1
  - 25%-50% = score 2
  - 50%-75% = score 3
  - 75%-100% = score 4

Gene expression in skin_qPCR

Log_2 of fold vs healthy

- Il17a and Il17f: analysed but very low expression or undetected.

- In skin of alopecic animals, Type I response related markers such as Ifng, Cxcl9, Cxcl10, Cxcl11 and Stat1 are increased, but also Il13, indicating Type 2 response involvement.

- Cd8 and Klrk1 (NKG2D) are also upregulated, according to the fact that CD8+NKG2D+ cells are major effectors in the human disease.

- There is good correlation between the most up and downregulated genes in AA patients and mice.

CD8+ cells in skin_IHC

Skin of non-alopecic C3H/HeJ — Skin of alopecic C3H/HeJ

Scale bar: 25 µm

CD8+ NKG2D+ in lymph nodes_Flow cytometry

*** 2-tailed unpaired t-test vs non-alopecic

- CD8+NKG2D+T cells have been reported to infiltrate hair follicles of AA patients and skin of alopecic mice.

- Accordingly, in our model we observe CD8+ cells infiltration in the skin of alopecic mice and increase of CD8+NKG2D+T cells in skin-draining lymph nodes.
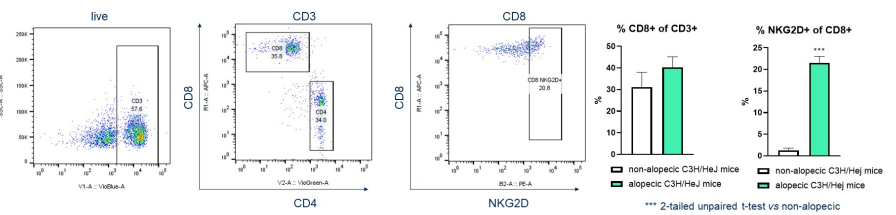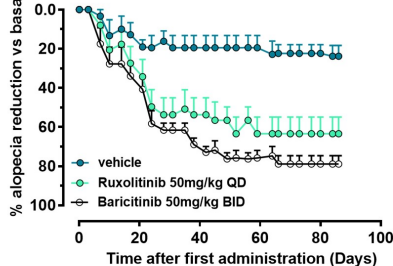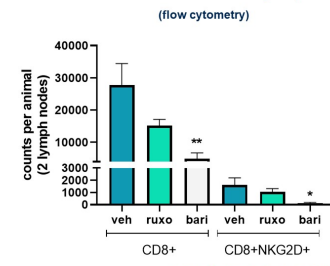
Effect of JAK inhibitors

- JAK inhibitors have been shown to be effective in AA patients, therefore in the pharmacological characterization of the model the effect of ruxolitinib and baricitinib was compared in terms of hair recovery, CD8+T cells infiltration, inflammation and gene expression profile in the skin.
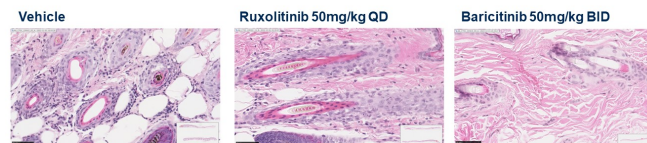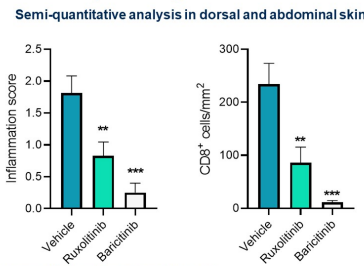
Clinical score


CD8+ cells infiltration in lymph nodes

(flow cytometry)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs Vehicle, by one-way ANOVA and Dunnett's post-hoc tests

Histopathological evaluation

Semi-quantitative analysis in dorsal and abdominal skin

Gene expression

Log_2 of fold vs healthy

Vehicle — Ruxolitinib 50mg/kg QD — Baricitinib 50mg/kg BID

Vehicle — Ruxolitinib 50mg/kg QD — Baricitinib 50mg/kg BID

- Both treatments reduced the alopecia score, CD8+ and CD8+NKG2D+ cells and expression of Cd8, Klrk1, Cxcl9, Cxcl10, Cxcl11, Cxcr3, Ifng and Il13, being baricitinib more effective.
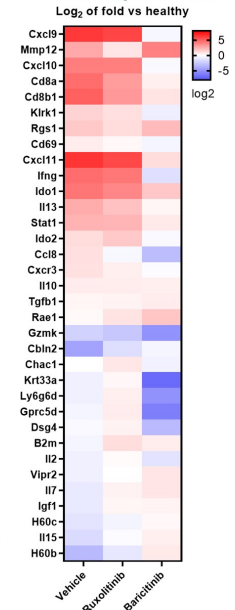
Conclusions

- Adoptive transfer of cultured lymph node cells from alopecic C3H/HeJ to naive mice induced alopecia in recipient animals.

- Inoculated mice show gene expression signature in skin similar to human disease. Type I response related genes such as Ifng, Cxcl9, Cxcl10, Cxcl11 and Stat1 are increased and also Type 2 related genes such as Il13. In addition, CD8+ cells infiltration is observed in alopecic skin as well as CD8+NKG2D+ cells increase in lymph nodes of affected animals.

- JAK inhibitors reduced the alopecia score and inflammation in the animals' skin, as well as AA related genes expression and CD8+ cells infiltration.

References:

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3. Hashimoto K et al. 2021 Jun;102(3):177-183. doi: 10.1016/j.jdermsci.2021.04.009