CHIEF SCIENTIST OFFICE

CODE: TCS/20/10

RESEARCH PROJECT BRIEFING

The immunology of alopecia areata: how do macrophages contribute to hair loss?

AIMS

Perform gene analysis via single cell sequencing on human alopecia areata (AA) scalp skin to uncover the role of macrophages in alopecia areata, specifically investigating how they are involved in causing and maintaining hair loss. Additionally, assess the distribution and proportion of macrophages in human alopecia scalp skin through fluorescent tissue staining, as well as determine their localisation with regards to hair follicles.

KEY FINDINGS

- Data characterising the immune cells in the blood of alopecia patients were published.
- Single-cell sequencing of human skin generated identifiable cell clusters.
- Using immunofluorescence microscopy of tissue staining, $CD68^{+}$ cells (macrophages) were located throughout the dermal layer of the skin and $CD163^{+}$ cells and $CD206^{+}$ cells (anti-inflammatory macrophages) were located near/surrounding hair follicles, and occasionally in the subcutaneous fat layer of the skin.
- Spatial transcriptomics gene sequencing was used to identify macrophages and other immune cells in the skin, at single-cell resolution, using the CosMX platform.
- Responses of monocytes from people with alopecia were examined, to assess how a clinically-used drug can affect myeloid cells and immune responses.

WHAT DID THE STUDY INVOLVE?

This study involved a number of key elements, designed to investigate the changes in the immune system of alopecia areata, with a focus on macrophages. Macrophages are cells of the immune system, which have important functions in the skin. They are closely related to monocytes, which are found in blood.

1) We had begun to analyse blood cell populations in people with alopecia areata. We initially worked to finalise these findings and published this work.
https://academic.oup.com/cei/article/210/2/175/6749633?

2) To investigate macrophage populations in the skin, we developed protocols to process biopsies of human skin into single-cells. This involved a large amount of optimisation. The single-cells were stained with fluorescent antibodies to label the cells and run through a cell sorter to separate immune cells and non-immune cells. Following this, these populations were used for gene analysis via single-cell RNA sequencing.

3) To identify the location of the macrophage populations in the skin of people with alopecia, we used fluorescence microscopy, CD68, CD163, and CD206 are molecules expressed by macrophages. We found that $CD68^+$ cells were located throughout the middle layer of skin called the dermis and $CD163^+$ cells and $CD206^+$ cells were located throughout the dermis, near/surrounding hair follicles, and occasionally in the deep layer of skin; subcutaneous fat layer. Initial results indicate that all these cells increase in the skin of people with alopecia.

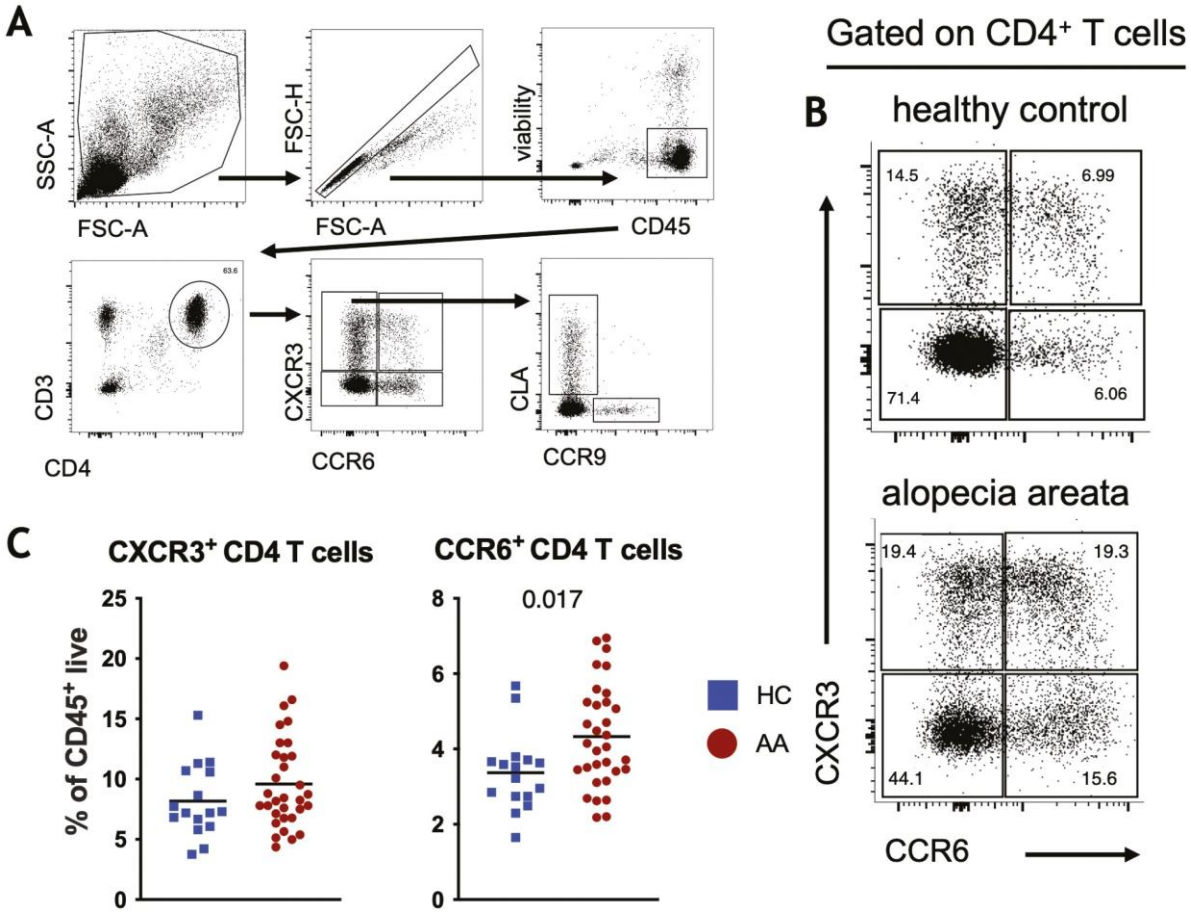
4) Spatial transcriptomics is an advanced technique, in which active genes can be identified. The platform we have recently acquired in Glasgow is called CosMX; this allows the measurement of 6000 genes at single-cell level. We have used the CosMX system to identify macrophages and other immune cells in the skin. We have generated data from both healthy and alopecia skin and will use this to compare macrophage locations, functions, and interactions with other cells.

5) To understand how the immune system could be affected by emerging alopecia treatments, we investigated their response to a drug that has recently completed phase II clinical trials. The results have shown significant effects on monocytes from the blood of people with alopecia, showing how effects on these immune cells are likely to be important in this context.

This project has enabled us to identify changes in the immune system that occur in people with alopecia areata, and we have now begun to understand how monocytes and macrophages may be targeted therapeutically in this common autoimmune condition.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN? CONTINUED

1) Analysis of blood cell populations in people with alopecia areata reveals changes in circulating T cells.
https://academic.oup.com/cei/article/210/2/175/6749633

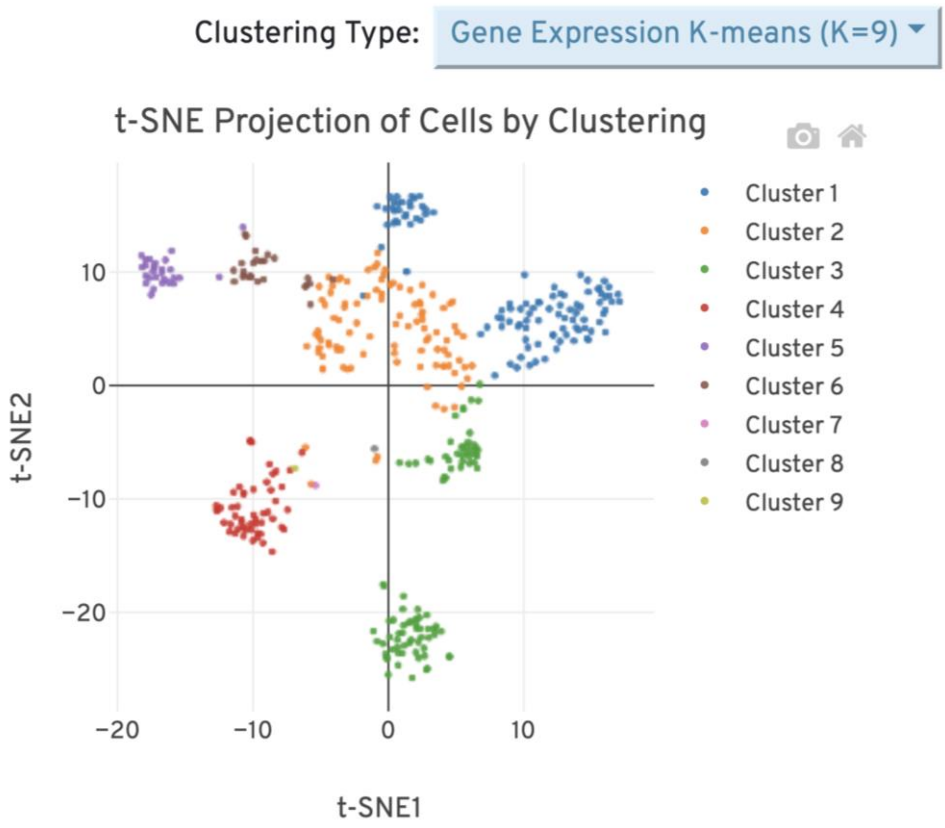


Clin Exp Immunol, Volume 210, Issue 2, November 2022, Pages 175–186

The figure is reproduced from the graphical abstract for the paper. It shows the how different populations of T cells were identified in our experiment(A), a representative example of how CD4 T cells differ between healthy controls and alopecia patients (B), and the collated data from our cohort (C). People with alopecia have higher frequencies of 'pathogenic' T cells, which express a molecule called CCR6.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

2) Initial single cell sequencing results from digested skin.



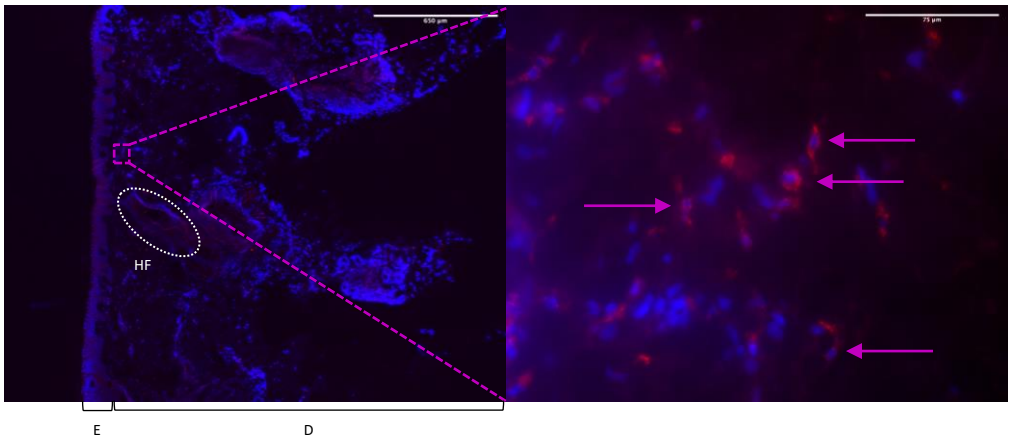
Fresh skin tissue was collected and used in over 20 experiments to optimize the number of cells recovered. Once optimized, a sample was processed and used to test the single-cell sequencing pipeline. The data shown are from these initial samples, demonstrating that at least nine clusters of cells can be identified by this method. We have secured additional funds to continue this work and will be integrating data from this technique with our spatial transcriptomics data (see below) to better understand the functions of macrophages and other cells in alopecia.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN? CONTINUED

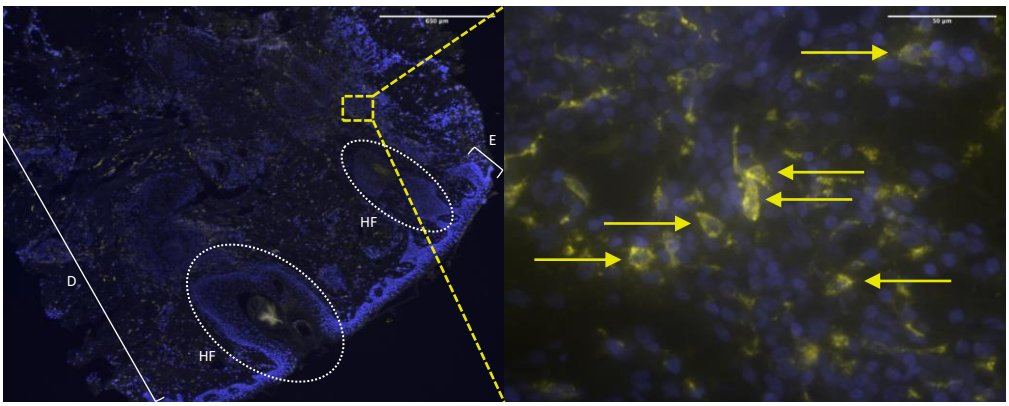
3) Immunofluorescence microscopy to identify macrophages in skin.

CD206 Staining on AA Skin

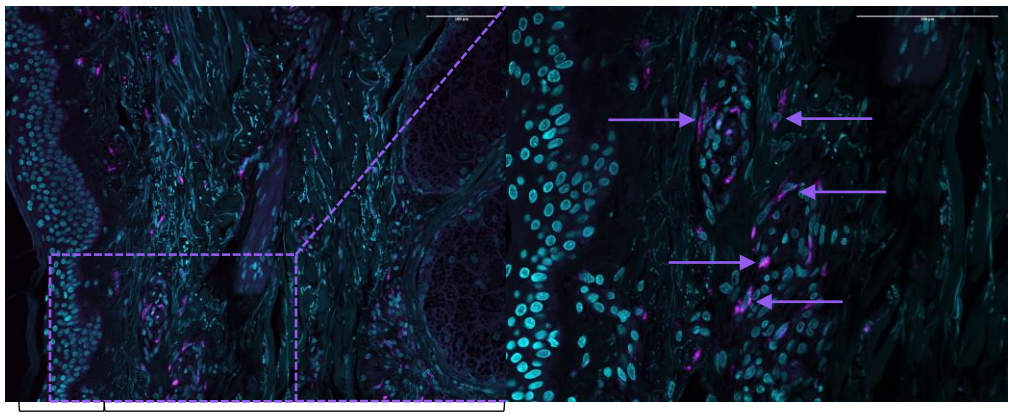
E = epidermis D = dermis HF = hair follicle



CD163 Staining on AA Skin

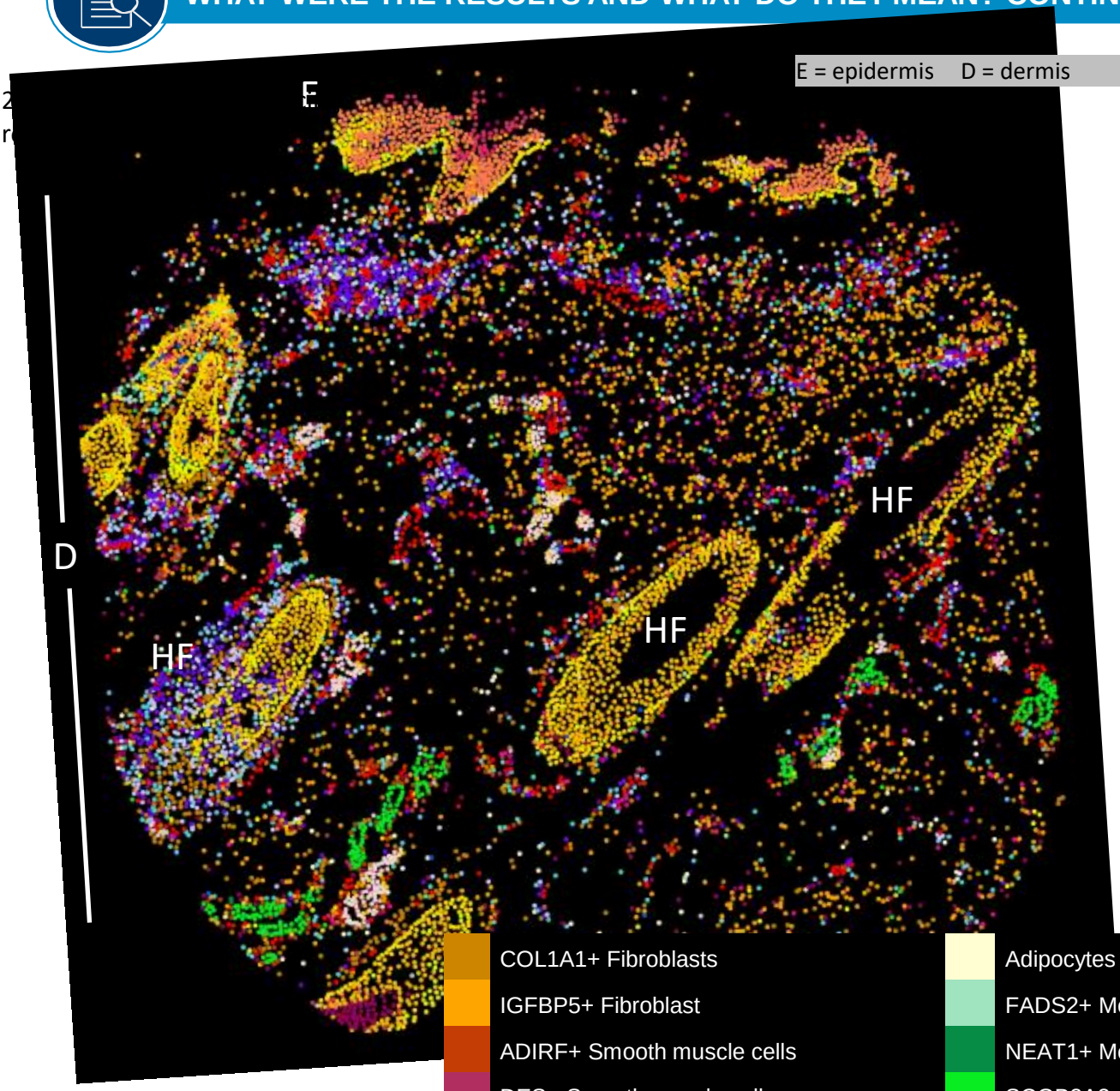


CD68 Staining on AA Skin



Fresh skin tissue was collected and used stained with fluorescent labels specific for the macrophage surface markers CD68, CD163 and CD206. Example images are shown here, revealing the locations of macrophages in the dermis in red (CD206, A), yellow (CD163, B) and pink (CD68, C). This technique will now be used on additional sections from healthy and alopecia skin to quantify changes found in alopecia, to help understand the roles played by macrophages in this condition.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN? CONTINUED

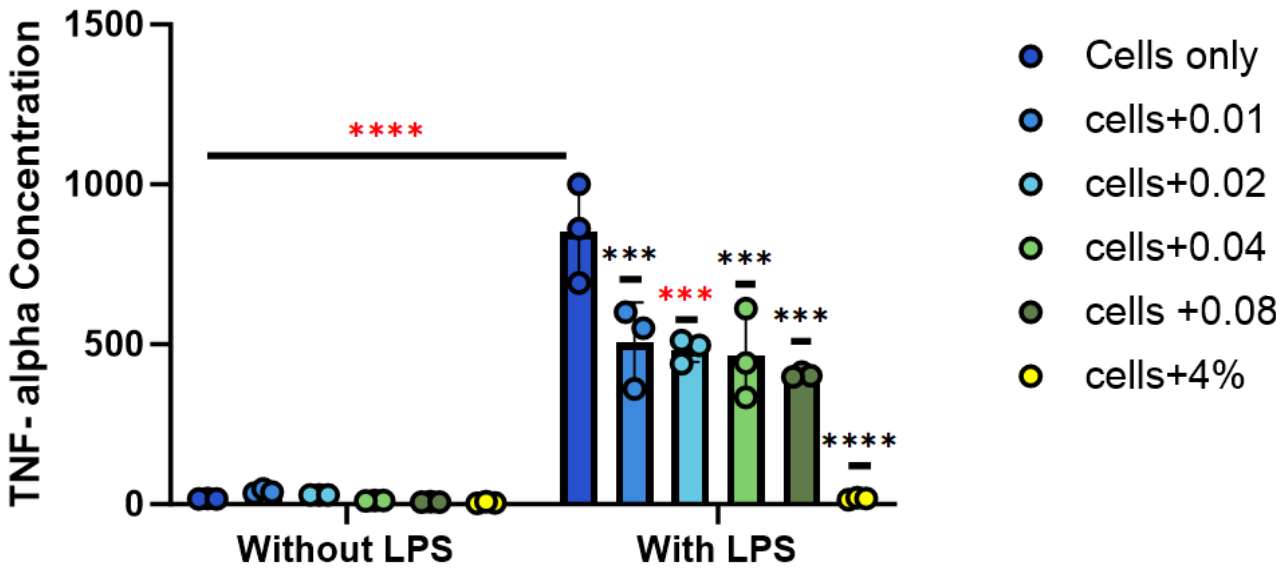


Fresh skin tissue was collected and analysed using CosMX to generate a gene map of the skin at single-cell resolution. Initial analysis reveals abundant macrophages in the dermis (as seen above), and alongside T cells in hair follicles with an over abundance of immune cells. Together, these data indicate the importance of macrophage-T cell interactions in alopecia.

- COL1A1+ Fibroblasts
- IGFBP5+ Fibroblast
- ADIRF+ Smooth muscle cells
- DES+ Smooth muscle cells
- AQP1+ Endothelial cells
- CCL21+ Endothelial cells
- Squamous epithelial cells
- CALML5+ Suprabasal Keratinocytes
- KRT1+PERP+ Suprabasal keratinocytes
- KRT17+ Keratinocytes
- SFN+ Keratinocytes
- KRT15+COL17A1+ Basal Keratinocytes
- Cycling cells
- Adipocytes
- FADS2+ Melanocytes
- NEAT1+ Melanocytes
- SCGB2A2+PIP+ Sebaceous
- Mast cells
- Macrophages
- Langerhans cells
- cDC
- T cells
- B cells
- NDUFA3+ unknown
- DISC1+ unknown
- Macrophage doublets

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN? CONTINUED

3) To understand how monocytes could be affected by emerging alopecia treatments,



Immune cells (PBMCs) were collected from the blood of healthy controls and from people with alopecia and were stored at -80ºC. Once thawed, they were cultured overnight in the absence or presence of lipopolysaccharide (LPS), which is known to activate monocytes. Blood monocytes are used here to provide an imperfect but widely-used model for the responses of macrophages in the skin.

We find that, as expected, LPS-activated blood cells produce a chemical, TNF-alpha. We also confirmed by flow cytometry that monocytes are the source of TNF-alpha in these cultures (not shown).

Importantly, non-toxic concentrations (0.01 – 0.08 ug/ml) of a medicine that has recently completed phase II trials in alopecia was added to cultures, and this significantly inhibited TNF-alpha production. In addition, the highest dose of the drug is cytotoxic.

These confidential data, generated in collaboration with the interested pharma, show how the actions of potential pharmaceutical agents can be modeled in vitro, to understand their function.

WHAT IMPACT COULD THE FINDINGS HAVE?

- These data may have the following impacts
- Identifying populations of cells from the blood of people with alopecia has demonstrated the possibility of categorising patients to better focus potential treatments.
- identifying the specific macrophages that contribute to alopecia may enable the development of better-targeted treatments.
- Demonstrating therapeutic function is enabling novel and effective therapies to gain access to the market, to help patients with alopecia as quickly and safely as possible.

HOW WILL THE OUTCOMES BE DISSEMINATED?

Part of this study is now published in a peer-reviewed journal. Unpublished aspects will also be submitted for peer review to be published in this way. Results will also continue to be presented at conferences and other scientific meetings, including in interactions with interested companies..

In addition, to allow access to affected individuals not within the scientific community, the results are also disseminated through Alopecia UK, both via the research blog on their website and at in-person events organised for the charity's members.

CONCLUSION

Human AA skin has increased proportions of macrophages compared to non-AA skin. We have generated data that will now be used to investigate these macrophages in more detail, and we have developed in vitro models to evaluate the effects of potential novel pharmaceutical treatments for this condition.

RESEARCH TEAM & CONTACT

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Additional Information

E.g.: Date the project was completed and the amount of funding received