Cytokines carried by plasma EVs as potential biomarkers predicting response to TNFi in PsA

Scientific abstract: Psoriatic Arthritis (PsA) is a chronic, heterogenous, inflammatory, immune-mediated disease characterised by multiple features and multi-comorbidities. The disease is difficult to diagnose because of the diverse symptoms and difficult to treat because of the complex inflammatory pathways involved in its development and progression. To this day, no curative treatment of PsA is available. Patients who do not respond to conventional therapies can be started on a Tumor Necrosis Factor inhibitor (TNFi). However, around 40% of patients with PsA respond poorly or fail to respond to this biologic treatment. TNF-α is the pro-inflammatory cytokine targeted by TNFi and is produced and released in the blood stream by immune cells such as T helper 1 (Th1) cells and monocytes. Immune cells signalling involved paracrine mechanisms via secretion of growth factor, cytokines, chemokines, and extracellular vesicles (EVs). EVs are a heterogeneous group of small membrane vesicles with key roles in cell-to-cell communication, cell signalling and immune response modulation. EVs contain a various proteins, lipids, cytokines, and nucleic acids collectively known as cargo. Cytokines can be released either in a soluble or EV-encapsulated form. We hypothesis that the level of cytokines carried inside EVs could predicts response to TNFi treatment. The overall aim of this project is to find a potential biomarker to predict the response to TNFi in PsA.

Lay abstract: Psoriatic Arthritis (PsA) is a disease that can cause pain, stiffness and swelling of joints and the spine. PsA is a form of inflammatory arthritis. This means it is thought to develop when the immune system, which is meant to protect you, attacks healthy cells in the body, especially around joints. In most cases, PsA occurs together with a skin disorder known as psoriasis. This disorder causes patches of red skin with silvery scales that can appear anywhere on the body. PsA affects 0.3 to 1% of the global population. Immune cells in PsA patients produce an excess amount of a protein called TNF which is responsible for the swelling and pain of joints. Anti-TNF, also known as TNF inhibitors (TNFi) are widely used as first-line targeted biologic treatments to reduce the inflammation. However, about 40% of PsA patients do not or only partially respond to this treatment. We try to find a molecule in the blood, known as a biomarker, to tell if TNFi will help the patient before they even take this drug. If the biomarker suggests the drug is not right for the patient, they can immediately try a more appropriate drug. One way the cells communicate is by releasing extracellular vesicles (EVs) which are small fatty structures containing anti- and pro-inflammatory molecules. In this project, we will analyse the contents of these EVs and find out if they can predict whether the patient will react positively to an anti-TNF drug.

Background: Psoriatic Arthritis (PsA) is a chronic, immune-mediated disease characterised by multiple features such as axial and peripheral arthritis, psoriasis, enthesitis, dactylitis and nail dystrophy (1). To this day, no curative treatment is available for PsA, and patients who do not respond to conventional therapies start on a TNF inhibitor (TNFi) (2). However, about 40% of PsA patients partially respond or fail to respond to this biologic drug (3), and there is a need for potential biomarkers predicting response to TNFi in PsA.

As part of our PARIS study (Psoriatic Arthritis – Resistance to TNF Inhibitors Study), we have recently published a review of cellular and molecular biomarkers of interest (4) to predict response to TNFi in PsA. After our extensive literature search, we recruited 18 patients with PsA and determined their response at 3 months after treatment using the psoriatic arthritis response criteria (PsARC) score (comprising a tender joint score (TJS), a swollen joint score (SJS) and a global health assessment of the physicist and patient (GHA)). Response was

defined by an improvement of at least 30% of the joint scores and an improvement of at least 1 point of the GHA. We further classified responders as either a good responder (0 TJS and 0 SJS) or a moderate responder (improvement of at least 30% of the joint score without reaching 100%).

Our first immunophenotyping results showed that the mean proportion of Th1 cells was significantly higher in good responders than in the moderate and non-responders (Figure 1A). Because the main pro-inflammatory cytokines released by Th1 cells are TNF-α and IFN-γ, we measured plasma levels of these cytokines and found their levels did not reflect the Th1 proportions. Mean plasma levels of TNF-α were similar in good and non-responders, and mean plasma level of IFN-γ was higher in non-responders than in good responders (Figure 1B).

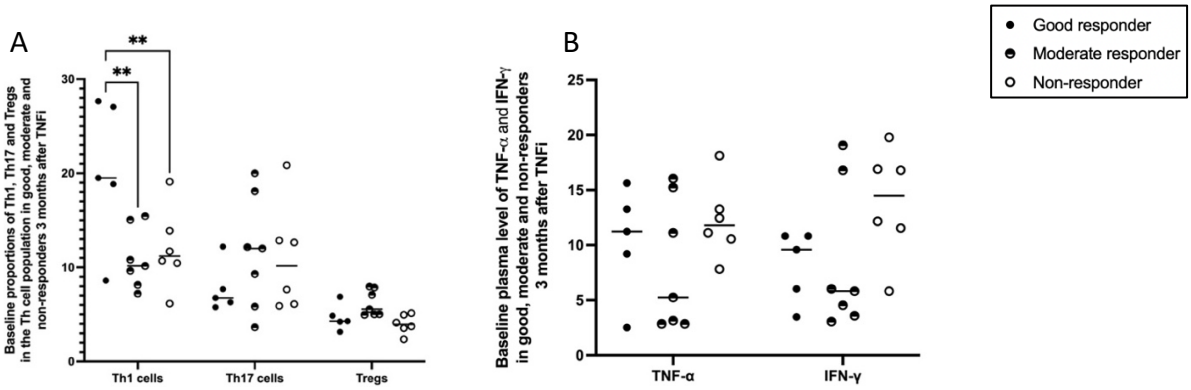

Figure 1: Unpublished data identifying different T helper immunophenotypes and cytokine profiles in good responder, moderate responder, and non-responder groups 3 months after TNFi injection.

Although plasma levels of pro-inflammatory cytokines measured in plasma may give a good indication of the immune pathways involved in each patient, but their levels overlap too much between responders and non-responders to allow their use as predictive biomarkers. However, besides circulating in the blood stream, cytokines can also be encapsulated in EVs (5). A potential explanation of the poor compatibility between Th1 cell levels and plasma cytokine levels may therefore be that Th1 cells secrete cytokines encapsulated in EVs.

Plasma EVs have been intensively studied in the last ten years and are increasingly recognized as promising circulating biomarkers for diagnosis, prognosis, disease progression, response to treatment and therapeutic drug delivery (6). EVs are membrane-bounded vesicles secreted by cells with a size ranging from 30 nm to over 1µm, and they contain proteins, mRNAs, miRNAs, lipids and cytokines as part of their cargo (6,7). Because of the role EVs play in cell-to-cell communication, they are suggested to be involved in inflammatory and autoimmune disorders (8,9). Not only do the levels of cytokines differ between EVs and plasma, they may also be better predictors of outcome. For instance, Zhang et al demonstrated that the mean level of TNF-α carried by EVs differs between non-progressive and progressive OA patients (10). In this project, we will verify if the level of cytokines carried inside EVs could predicts response to TNFi treatment.

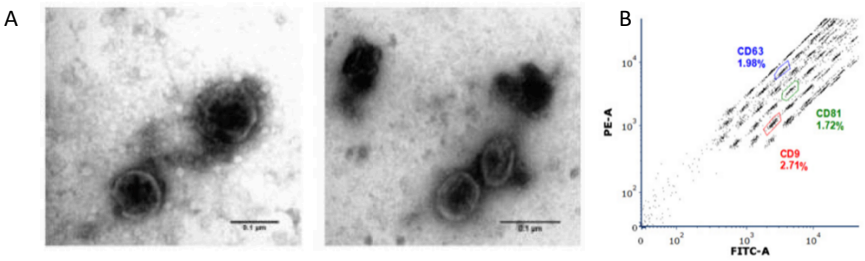

Figure 2: EV characterisation. A-EV characterisation by TEM. B-Representa-tive flow cytometry analysis of EV prepara-tion using the MACS-Plex exosome detection kit from Miltenyi (7,11).

Previous research from our lab focused on charactering size, distribution and immunosuppressive properties of EVs derived from bone marrow and umbilical cord mesenchymal stromal cells, and demonstrated their role in rheumatoid arthritis (Figure 2) (7,11). We also evidenced that environment (normoxia/hypoxia, pro-inflammatory priming) could alter the EV protein cargo profile (7) and that they may have potential as a cell-free therapy (12).

Methods:

Sample collection: Informed consent has already been obtained from patients and healthy donors who enrolled in the PARIS study. Freshly collected blood samples have been centrifuged and the resulting plasma samples stored at -80°C.

EV isolation: EVs will be isolated from plasma using the ExoQuick-LP kit (System Bioscience Inc.). The EV pellet and EV-depleted supernatant will be stored in -80°C. Isolation of plasma EVs is a complex process. The minimal information for studies of extracellular vesicles (MISEV) guidelines will be followed such as removing cells and large proteins by ultracentrifugation, avoiding freeze/thaws cycles, and performing additional filtration steps to deplete residual platelet which are the same size as EVs (13).

EV characterisation and cytokine profiling: After a sonication of the EV samples to release cargo proteins and surface proteins, a Bicinchoninic Acid (BCA) assay will be performed to measure total protein concentration. Nanoparticle tracking analysis (NTA) of isolated EV and EV-depleted plasma will be performed to determine concentration and particle size distributions. Transmission electron microscopy (TEM) will be used to obtain images of EVs and determine their morphology. Flow cytometry will be used to identify characteristic tetraspanins surface markers using MACSPlex exosome detection kit (Miltenyi). Cytokine levels will be measured in EV pellet (endo-EV) and in EV-depleted plasma (exo-EV) using multiple ELISA assays.

Statistics: Cytokine levels will be checked for normality and log-transformed if needed. Paired t-tests will be used to compare cytokine levels between plasma and EVs, independent t-tests to compare between healthy donors and PsA patients, 1-way ANOVA to compare between good, moderate, and non-responders, and Spearman's correlation coefficient to correlate between cytokine levels in EVs and proportion of Th1 cells. All statistical analyses will be performed using GraphPad Prism, using a two-sided p-value below 0.05 to denote significance.

Expected results:

In this study:

- We will determine whether the cytokine levels in EV lysates is different in PsA patients and healthy donors, as well as in responders and non-responders to TNFi.
- We will also reveal if the cytokine levels differ in lysate of EVs isolated form plasma, and in EV-depleted plasma.
- We will verify if the level of TNF-α and IFN-γ measured in EV lysates can be correlated to the proportion of Th1 cells.

Significance for psoriatic arthritis:

The findings from this study will hopefully help clinicians to select a more precisely targeted biologic therapy among approved drugs such as TNFi, IL-17i, IL-23/12i or JAKi. This will allow immediate treatment with the most appropriate drug rather than following the usual default cascade of drugs starting with TNFi and in the case of non-response followed by trialling the other treatments mentioned. This will save money, but more importantly will save patients the time wasted on taking inappropriate drugs, resulting in better treatment outcomes and quality of life.

References

1. Gottlieb AB, Merola JF. Axial psoriatic arthritis: An update for dermatologists. J Am Acad Dermatol. 2021 Jan;84(1):92–101.

2. Gossec L, Coates LC, de Wit M, Kavanaugh A, Ramiro S, Mease PJ, et al. Management of psoriatic arthritis in 2016: a comparison of EULAR and GRAPPA recommendations. Nat Rev Rheumatol. 2016 Dec;12(12):743–50.

3. Veale DJ, Fearon U. The pathogenesis of psoriatic arthritis. Lancet. 2018 Jun 2;391(10136):2273–84.

4. Makos A, Kuiper JH, Kehoe O, Amarasena R. Psoriatic arthritis: review of potential biomarkers predicting response to TNF inhibitors. Inflammopharmacology. 2023 Feb;31(1):77–87.

5. Sass D, Fitzgerald W, Wolff BS, Torres I, Pagan-Mercado G, Armstrong TS, et al. Differences in Circulating Extracellular Vesicle and Soluble Cytokines in Older Versus Younger Breast Cancer Patients With Distinct Symptom Profiles. Front Genet. 2022;13:869044.

6. Bettio V, Mazzucco E, Antona A, Cracas S, Varalda M, Venetucci J, et al. Extracellular vesicles from human plasma for biomarkers discovery: Impact of anticoagulants and isolation techniques. PLoS One. 2023 May 10;18(5):e0285440.

7. Hyland M, Mennan C, Wilson E, Clayton A, Kehoe O. Pro-Inflammatory Priming of Umbilical Cord Mesenchymal Stromal Cells Alters the Protein Cargo of Their Extracellular Vesicles. Cells. 2020 Mar 16;9(3):726.

8. Born LJ, Khachemoune A. Extracellular vesicles: a comprehensive review of their roles as biomarkers and potential therapeutics in psoriasis and psoriatic arthritis. Clin Exp Dermatol. 2023 Mar 22;48(4):310–8.

9. Lu M, DiBernardo E, Parks E, Fox H, Zheng SY, Wayne E. The Role of Extracellular Vesicles in the Pathogenesis and Treatment of Autoimmune Disorders. Front Immunol. 2021 Feb 24;12:566299.

10. Zhang X, Hsueh MF, Huebner JL, Kraus VB. TNF-α Carried by Plasma Extracellular Vesicles Predicts Knee Osteoarthritis Progression. Front Immunol. 2021;12:758386.

11. Kay AG, Treadwell K, Roach P, Morgan R, Lodge R, Hyland M, et al. Therapeutic Effects of Hypoxic and Pro-Inflammatory Priming of Mesenchymal Stem Cell-Derived Extracellular Vesicles in Inflammatory Arthritis. Int J Mol Sci. 2021 Dec 23;23(1):126.

12. Hyland M, Mennan C, Davies R, Wilson E, Tonge DP, Clayton A, et al. Extracellular vesicles derived from umbilical cord mesenchymal stromal cells show enhanced anti-inflammatory properties via upregulation of miRNAs after pro-inflammatory priming. Stem Cell Rev Rep. 2023 Oct;19(7):2391–406.

13. Welsh JA, Goberdhan DCI, O'Driscoll L, Buzas EI, Blenkiron C, Bussolati B, et al. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. J Extracell Vesicles. 2024 Feb;13(2):e12404.