HELICAL

H2020-MSCA-ITN-2018-813545

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Health Data Linkage for Clinical Benefit

Deliverable D3.7

WP3 Summary Report

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 813545.

Work package 3 Linkage of clinical data to plasma and tissue analyses

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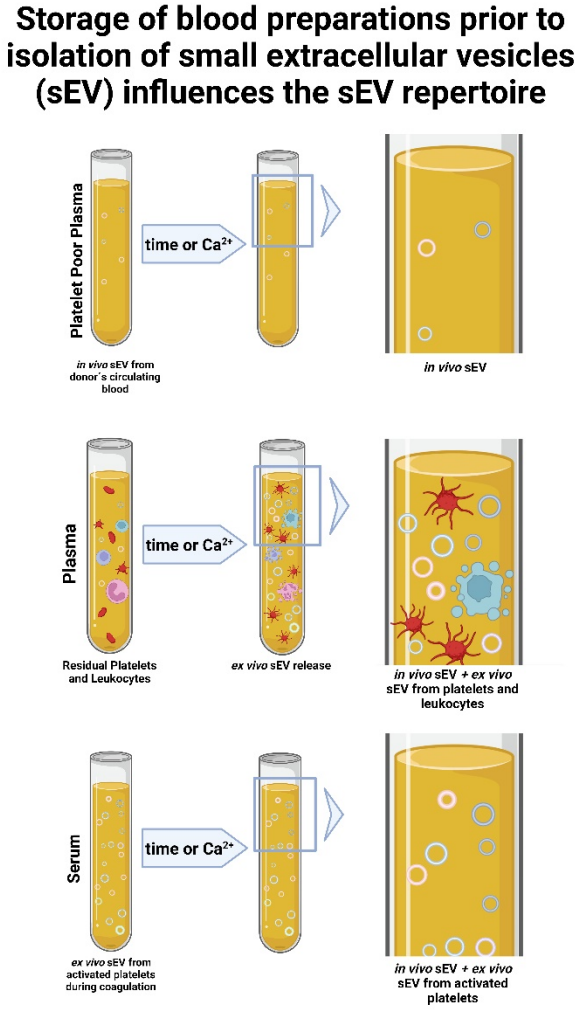
AIM: WP3 uses novel techniques to correlate results from proteomic and morphometric analyses of plasma and tissue with clinical outcome, aiming at stratifying patients with vasculitis according to disease activity, while building on the overall HELICAL theme of ethical linkage of experimental and clinical data to improve patient care. Using existing resources established in past and ongoing collaborations, the common goal of the four ESR projects is to develop clinical tools, supported by machine learning algorithms, that assist clinicians in diagnosing active vasculitis and predicting outcome. Extracellular Vesicles (EV) are derived from activated resident or circulating cells and carry a cargo of (surface) proteins, DNA and RNA that they deliver to other cells. In autoimmune diseases like AAV they are produced by endothelial cells and leukocytes in response to injury, with the specific vascular beds affected by this condition producing a different pattern of EVs. One aim of this WP is to develop and optimise techniques to identify the source of EVs and establish them as a diagnostic and predictive tool in AAV using consortium samples. Adopting an alternative strategy, we will exploit the increasing evidence from large multinational consortia like Neptune and INTEGRATE that have defined tissue morphological changes (descriptors) that can be used as predictors of outcome in renal ANCA vasculitis. However, analysis and validation of these descriptors is time consuming, hindered by inter-observer variation and by difficulties in interpreting them due to clinical outcome data variability. Based on existing descriptors and algorithms, this project aims to define morphological changes in renal biopsies from patients with ANCA vasculitis that are suited to automated morphometric analysis and subsequent validation using existing clinical outcome data. In parallel, we aim to investigate novel autoantibodies to combinations of peptides and EV proteins with a view to diagnosing vasculitis flare and defining flare risk. Part of this aim is linked to a novel test developed by SME Firalis (FI), based on an innovative set of selective soluble biomarkers that will be combined with an algorithm based on the patients' clinical data, and a web-based user interface to facilitate diagnosis of active vasculitis. 30 biomarkers have been distilled to 4 key peptides, which display excellent biomarker characteristics. The aim of this project is to take this approach to Technology Readiness Level (TRL) 4 in the path of commercial test development.

EXPERIMENTS CONDUCTED DURING THE SECOND HALF OF THE ACTION

The importance of the pre-analytic pathway when studying sEV

ESR11 (MUW) worked on optimizing methods to purify and measure sEV from human blood and establish them as markers for AAV. Standardised procedures, including complete depletion of residual blood cells, are indispensable for obtaining reliable results from sEV circulating in the blood stream. The isolation of sEVs requires appropriate methods to avoid contamination, and differential ultracentrifugation (DUC) resulted as the most robust method for sEV isolation. Serum stored at -80 was identified as the most appropriate sample type for sEV studies. The results also identified potential sources of variation, highlighting the need for strict pre-analytical protocols. For sEV biomarker discovery, stored platelet poor plasma (PPP) was identified as the best sample type for sEV biomarker discovery studies, facilitating the quantification of sEV from retrospectively characterized and biobanked sample cohorts. Stored plasma and serum are less suitable for sEV studies.



Use of sEV to define organ-specific disease activity in AAV

ESR11 also worked on the development of a custom microarray for high-throughput analysis of sEV from the blood of patients with ANCA-associated vasculitis (AAV). The approach did not generate reliable or consistent results, possibly due to the high degree of glycosylation of the sEV surface proteins making antibody binding sites inaccessible. Additional approaches

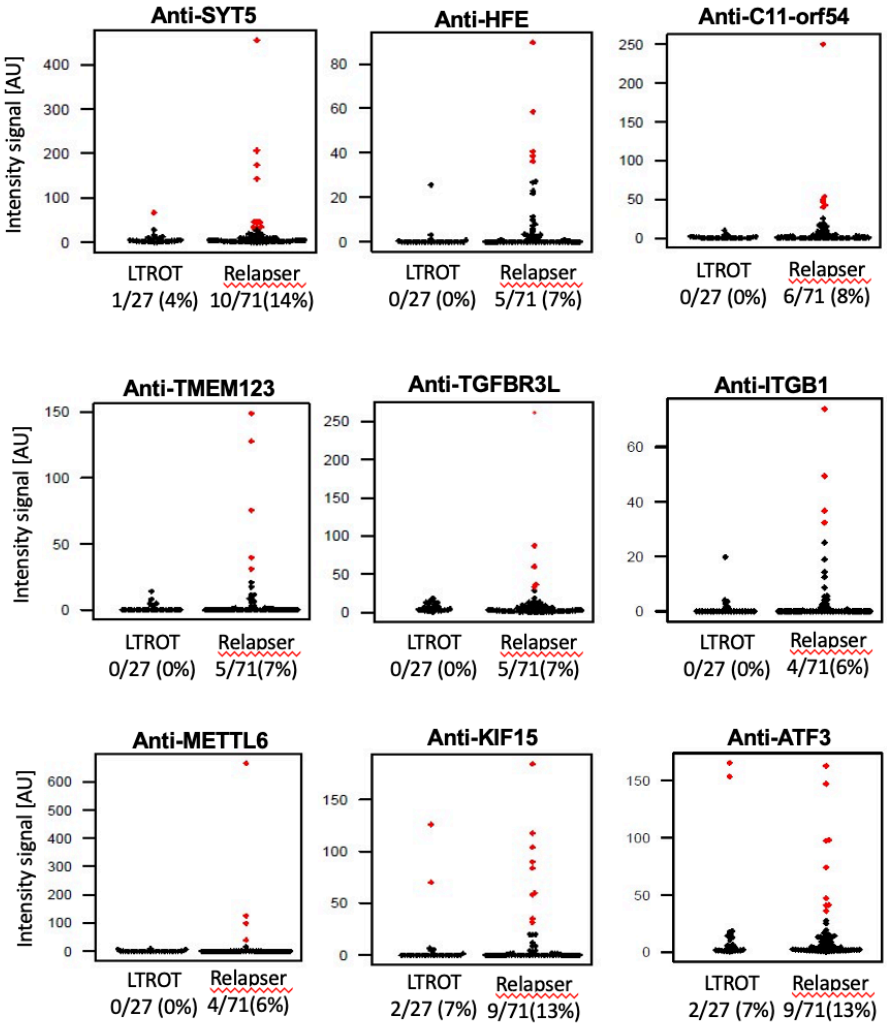
(i.e. enzymatic removal of glycans, heat and chemical pre-treatment) are being tested to improve the reliability of sEV profiling using antibody bead-arrays.

Automated analysis of renal histopathology

ESR 12 (TG) worked on developing and testing machine learning models that can identify features in biopsies of patients with AAV and assessing their suitability in predicting patient outcomes. Initially, a U-net model was used to identify glomeruli in kidney biopsies. However, the model´s performance suffered when dealing with images containing cropped glomeruli. For this reason, the model was switched to the YOLO model, which showed to be faster and better suited for clinical use to identify PAS-stained whole slide images (WSIs). The YOLO model was trained to classify glomeruli as normal, crescentic or sclerosed. The model has been refined using a large number of WSIs from Trinity College, with linkage to granular longitudinal data to correlate with clinical outcomes. Further development of a multiple instance model (MIL) for clinical data is ongoing to predict outcomes and a framework to develop ore user friendly machine learning models.

Proteomic and transcriptomic profiling of peripheral blood to define AAV disease activity and predict relapse

ESR13 (KTH) and ESR 14 (Firalis) has been working on the serum/plasma cohort from the Irish Rare Kidney Disease (RKD) Registry and Biobank to identify autoantibodies and mRNA markers as predictors of relapse. **ESR13 (KTH)** performed **autoantibody profiling** of 98 samples collected at remission from patients classified as LTROT and relapsers by using **proteome-wide antigen array technology** and optimized workflow developed at the Protein Science Department at KTH. Nine autoantibodies with higher reactivity were observed in subgroups of samples collected from AAV patients experiencing subsequent relapse (see figure below). These bind to activating transcription factor 3 (ATF3), methyltransferase-like protein 6 (METTL6), synaptotagmin 5 (SYT5), homeostatic iron regulator (HFE), chromosome 11 open reading frame 54 (c11orf54), transmembrane protein 123 (TMEM123), transforming growth factor beta receptor 3 like (TGFBR3L), integrin subunit beta 1 (ITGB1), and kinesin 15 (KIF15). These findings are under verification for their potential to predict future relapses as single autoantibodies or autoantibody panel(s).



ESR14 (Firalis) applied the **BIOPRED RNA profiling technology**, developed to identify mRNA targets associated to autoimmune-inflammatory disorders such as rheumatoid arthritis and lupus, on the cohort from the Irish Rare Kidney Disease (RKD) Registry and Biobank to identify new transcriptomic biomarkers able to predict relapse. The technology is based on a targeted gene sequencing panel of 2155 mRNA targets associated with inflammatory and pro-inflammatory pathways, immune response pathways, interleukins, chemokines, growth factors, metalloproteinases, and more. Incorporating the 2155 mRNA from the BIOPRED panel into a logistic regression model significantly improved the accuracy of predicting different AAV clinical stages (AAV active, remission, LTROT, and relapse) and separating them from healthy individuals. The findings therefore suggest that the BIOPRED panel could be used as a precision medicine tool for diagnosing active vasculitis (TRL3). We went on to assess whether the peripheral transcriptomic profile could predict flares, allowing adjustment of medication dosage and developing personalized treatment. Here, the results were more

subtle, the study under-powered and the assay not currently ready for taking forward for this diagnostic indication (TRL2). Further validation in additional patient cohorts and prospective studies are needed.

KEY SCIENTIFIC OUTPUTS:

- Optimization of methods for purifying and measuring sEVs from human blood.
- Ongoing development of a custom microarray for high-throughput analysis of sEVs from AAV patients.
- Development of machine learning models to identify features in biopsies of AAV patients and predict patient outcomes.
- Identification of 9 autoantibodies at high reactivity in subgroups of patients that will experience relapses.
- A proof-of-concept study showing the potential of the BIOPRED assay in separating active from remission vasculitis, and in predicting AAV relapse.

Published manuscripts:

- Małys, M. S. S., Aigner, C., Schulz, S. M. M., Schachner, H., Rees, A. J. J., & Kain, R. (2021). Isolation of Small Extracellular Vesicles from Human Sera. *International journal of molecular sciences*, *22*(9), 4653. https://doi.org/10.3390/ijms22094653
- Małys et al. Small extracellular vesicles are released *ex vivo* during coagulation from activated platelets and during storage from residual blood cells. Submitted to Journal of Extracellular Biology on the 29.03.2023.

Manuscripts in preparation:

- Bayati S, Scott J, Little M, Nilsson P, Pin E. Identification of autoantibodies for the prediction of relapses in ANCA-associated vasculitis.
- Mescia F, Bayati S, Brouwer E, Rees A, Kain R, Lyon P, Nilsson P, Pin E. Anti-kinesin antibodies associated to ANCA-vasculitis. To be submitted to the International Journal of Molecular Sciences.
- Matthieu Coq, Yagmur Dogay, Gisella Pattarone, Huseyin Firat, Jennifer Scott, Caitriona McEvoy, Mark A Little. Quantification of ANCA vasculitis disease activity and relapse risk using peripheral blood transcriptomic profiling. To be submitted to Arthritis and Rheumatology.