HFGPR
Human Functional Genomics Project Romania

Human Functional Genomics Romania CONFERENCE

PROGRAM & ABSTRACTS

2 days conference
OPEN DOORS AT 08:30 AM

SEPTEMBER 3-4th 2025

Hotel Napoca, Cluj-Napoca, Romania

Organizer: HFGP Romania

PROGRAM SUMMARY

Wednesday 3 September

8.45 Welcome Mihai Netea: what is Human Genomics Project Romania

Basic mechanisms in regulating immune responses
Chaired by: **Tania Crisan and Martin Jaeger**

9.00 **Musa Mhlanga**
Epigenetic control of immune responses

9.30 **Collins Boahen**
Genetic regulation of immune responses

10.00 **Yang Li**
Complex regulation of immune responses

10.30 Break

11.00 **Quirijn de Mast**
Dietary shifts and immune responses

11.30 **Sarah Walmsley**
Trained immunity in respiratory infections

12.00 **Andrian Fratea**
Dual Transcriptome–Proteome Analysis Reveals Distinct Immune Endotypes in Sepsis

12.15 **Emmanoil Stylianakis**
Molecular pathways affected by clarithromycin treatment in community-acquired pneumonia Insights from the ACCESS trial

12.30 Lunch and poster session

Trained immunity: the innate immune memory of host defense
Chaired by: **Medeea Badii and Athanasios Ziogas**

14.00 **Georgiana Cabau**
Serum Metabolomics Reveals Dyslipidaemia in Gout and Hyperuricemia - Exploring Inflammatory Links Through Integrative Multi-Omics

14.15 **Jeroen Deckers**
Overcoming immune suppression in ovarian cancer by reprogramming central innate immune memory

14.30 **Anca Riza**
Genetic regulation of immune responses

15.00 **Andre van der Ven**
Systems immunology in HIV

15.30 Break

16.00 **Maziar Divangahi**
Trained immunity in tuberculosis

16.30 **James Cheng**
Synergy Among Epithelial, Monocyte, and CD8 T Cell Triad Enhances Antiviral Immunity

17.00 **George Hajishengalis**
Myeloid cell reprogramming in chronic inflammation

18.00 On own for dinner, Speaker dinner

Thursday 4 September

Systems immunology in health and disease (1)
Chaired by: **Anca Riza and Julia van Heck**

8.45 **Tania Crisan**
Immune dysregulation in gout

9.15 **Niels Riksen**
Inflammation and atherosclerosis

9.45 **Qiuyao Zhan**
Dynamic single-cell multiomics Signatures Define Three Distinct Trained Immunity Programs in Long COVID

10.00 **Prashant Changoer**
Immune modulation by lenvatinib in patients with non-medullary thyroid cancer

10.15 Break

10.45 **Marcellus Korompis**
Enhancing Tuberculosis Vaccine Efficacy with a Heterologous mRNA-ChAdOx1 Prime-Pull Strategy Targeting Lung-Resident Memory T Cells

11.00 **Emil Vorsteveld**
Long and short read transcriptomics reveals novel genes and transcripts in the human immune response

11.15 **Ioana Berindan-Neagoe**
Molecular traffic through tumor microenvironment

11.45 **Kamal Khanna**
Antiinflammatory innate immune memory

12.15 Lunch and poster session

Systems immunology in health and disease (2)
Chaired by: **Orsolya Tokes Gaal and Jessica dos Santos**

13.30 **Cristian Coarfa**
A Multi-omic Framework for Personalized Medicine in Tuberculosis

14.00 **Reinout van Crevel**
Early TB phenotypes – a mechanistic perspective

14.30 **Hang Korng Ea**
Fasting and inflammation in gout

15.00 Break

15.30 **Irina Udalova**
Neutrophils: function and development

16.00 **Triantafyllos Chavakis**
Keynote: Trained immunity as a treatment approach in cancer

16.45 **Farewell and prize winners**

19.00 Conference dinner & drinks

ABSTRACTS

Selected oral presentation and posters

TRANSGENERATIONAL TRANSMISSION OF TRAINED IMMUNITY IN MICE: INFLUENCE OF PATERNAL IMMUNE TRAINING DURING PREGNANCY

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Objectives
Emerging evidence suggests that trained immunity, also referred to as innate immune memory, can be transmitted across generations in mammals, primarily via epigenetic modifications in innate immune cells. Notably, alterations in sperm DNA methylation following immune challenges have been implicated in this process. This study investigates whether the cross-protective effects of trained immunity can be transmitted from male mice to their offspring through transgenerational immune priming and whether the developmental timing of paternal immune training-specifically during puberty-modulates the inheritance.

Materials
Male C57BL/6 mice from F0 generation were divided into two age groups (n=10mice/group) pre-pubertal (4 weeks old) and adult (18 weeks old). Each group received an intravenous inoculation of a sub-lethal dose of Candida albicans to induce trained immunity. Control groups received an equivalent volume of phosphate-buffered saline (PBS). Six weeks post-inoculation treated and control males were mated with naïve wild-type females to generate the F1 generation. Upon reaching sexual maturity, F1 males were similarly bred with wild-type females to produce F2 offspring. To assess transgenerational immune effects, both F1 and F2 offspring were challenged with Escherichia coli and lipopolysaccharide (LPS). Microbiological and immunological analyses were performed, including quantification of colony-forming units (CFUs) in target organs (liver, kidney, lung) and survival rates were monitored.

Results
No mortality was observed in the F1 offspring of Candida inoculated fathers, whereas a 17% mortality rate was recorded in the control groups for both pre-pubertal and adult stages ($p=0.16$). A statistically significant reduction in CFUs was observed in the liver, kidney and lungs of offspring from trained fathers compared to controls in both age groups ($p<0.05$), suggesting enhanced pathogen clearance.

Conclusions
These findings underscore the potential role of epigenetic modifications in the paternal germline as a mechanism for transmitted trained immunity and shaping immune competence in descendants.

Keywords
trained immunity, innate immune memory, transgenerational inheritance, epigenetic modifications, paternal immune priming

References

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A MULTI-OMIC FRAMEWORK FOR PERSONALIZED MEDICINE IN TUBERCULOSIS

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Objectives
Tuberculosis (TB) is heterogenous disease and applying host directed therapies in a one-size-fits-all approach is not effective. We are presenting and endotype approach to support precision medicine in TB.

Materials
We previously determined putative TB endotypes by mining publicly available blood transcriptomics, identifying two major endotypes A and B, each with two sub-endotypes, A1/A2 and B1/B2. By using machine learning and a validation cohort, we determined that patients with endotype A had a lower cure rate and higher time to culture conversion, and also higher scores for signatures of TB diseases severity and treatment failure. Endotype A had higher TNFA and interferon gamma signaling, whereas endotype B had higher MTORC1 and MYC signaling. The two endotypes had opposite correlation with multiple medication signatures. Signature activity scores were used to understand TB associated commorbidities and lung function after treatment completion.

Results
New analysis demonstrates that endotype A has increased enrichment for myocardial and cerebral ischemia, lung cancer, and pulmonary fibrosis, whereas those with endotype B were at higher risk for Asthma.
Integrating with a sepsis framework, we determined that patients with endotype A had higher scores for systemic inflammation, whereas those with endotype B had higher score for immune induced killing capacity. On a different biological dimensions, epigenomics, our group showed that DNA hypermethylation persists over 12 months from TB treatment completion. Trying to understand why certain patients, despite successful therapy, have worsened lung function, we conducted DNA methylation analysis in a cohort of 68 patients from South Africa, with PFT including FEV1 and FVC measured. To our surprise, we observed increased DNA methylation in immune system pathways correlated positively with increased lung function, however, hypomethylation of the same pathways associated with fast pathogen killing capacity, indicating a epigenetic tradeoff. Using WGCNA network analysis we uncovered a hypermethylated gene-enhancer network associated with improved lung function.

Conclusions
Our work showed that TB endotypes are promising as a tool to understand the heterogenous immune response during TB, specifically with the aim of identifying clinically relevant endotype-specific means to mitigating TB associated pathology. Future efforts involve deep omics, PFT and immune characterization of each endotype, and consider the tradeoffs for each endotype between pathogen killing and lung function post treatment.

Keywords
Tuberculosis, endotypes, epigenetic tradeoff, post-TB lung disease

References

DiNardo AR, Gandhi T, Heyckendorf J, Rajapakshe K, Grimm SL, Nishiguchi T, Reimann M, Kahari J, Dlamini Q, Lange C, Goldmann T, Marwitz S, Cirilo JD, Kaufmann SHE, Netea MG, Crevel RV, Mandalakas AM, Coarfa C. Gene expression signatures identify biologically and clinically distinct endotypes in Tuberculosis. Eur Respir J. 2022 Feb 15. PMID: 35169026.

PROTEOMIC SIGNATURE IN HYPERURICEMIC PEOPLE LIVING WITH HIV

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Objectives
People living with HIV (PLHIV) under viral remission following treatment with retro viral therapy (ART) are exposed to persistent inflammation, which increases their susceptibility to non-AIDS comorbidities. Elevated uric acid levels, often associated with inflammation and metabolic dysregulation, may play a key role in exacerbating these conditions and contributing to chronic inflammatory profile. This study investigates the proteomic signature of hyperuricemia in virally suppressed PLHIV.

Materials
We analyzed data from an existing group comprising of 1,866 participants from the 2000HIV cohort, a Dutch multi-center, longitudinal study in virally suppressed PLHIV using antiretroviral therapy (ART). Biological sample collection and initial laboratory assessments were conducted at the time of study inclusion, followed by additional analyses of urate and creatinine levels in both plasma and urine. Circulating concentrations of targeted proteins (n=2367) (Olink® Explore Panel) were compared between PLHIV with vs. without hyperuricemia (7 mg/dL).

Results
The study included 1,866 PLHIV, comprising 188 individuals with hyperuricemia and 1,678 normouricemic controls (NU), indicating that 10.1% of the cohort had hyperuricemia. Among the 2,367 proteins analyzed, we identified 365 differentially expressed proteins (DEPs) between the two groups. The most upregulated proteins were OXT (oxytocin), followed by IGSF9 (immunoglobulin superfamily member 9) and FGF-21 (fibroblast growth factor 21). In contrast, the top 3 downregulated proteins were SHBG (sex hormone-binding globulin), followed by UMOD (uromodulin) and ADIPOQ (adiponectin).

Conclusions
Hyperuricemic PLHIV display distinct proteomic signatures, potentially driven by a urate-dependent effect that promotes inflammation in asymptomatic hyperuricemia. The interplay between urate and HIV could be mediated through metabolic, renal, and inflammatory pathways, with ART playing a variable role. Further research may provide deeper insights into the role of urate in non-AIDS comorbidities among PLHIV.

Keywords
Uric acid, Hyperuricemia, Chronic inflammation, Proteomic signature

DYNAMIC SINGLE-CELL MULTIOMICS SIGNATURES DEFINE THREE DISTINCT TRAINED IMMUNITY PROGRAMS IN LONG COVID

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Objectives
To determine whether trained immunity persists in long COVID and to characterise its cellular heterogeneity and underlying epigenetic mechanisms.

Materials
Blood sample was obtained from acute-COVID, Long-COVID (≥4 weeks post-infection with fatigue/respiratory symptoms) and recovered donors (2020-2022, Hannover). PBMCs were Ficoll-isolated and nuclei subjected to 10x single-nucleus multiome sequencing (snRNA + snATAC). Pseudomonas aeruginosa–restimulated aliquots were profiled in parallel. Donor genotypes (Illumina GSA v3, TOPMed-imputed) enabled SNP-based demultiplexing (Souporcell). After stringent QC, libraries were integrated with Seurat/Signac, clustered, and annotated. Differential expression/accessibility, peak-to-gene linkage, chromVAR TF-activity scoring and CellChat signalling inference were performed with standard parameters. Trained-immunity (TI) states were defined from COVID-19 vs recovered individuals and long COVID vs. recovered individuals. AUCell scoring of TI signatures mapped subclusters across cohorts. Pathway enrichment employed GSEA (clusterProfiler, MSigDB H/C5 + Reactome).

Results
Here, we profiled peripheral blood mononuclear cells from COVID-19 patients, LC patients with fatigue and respiratory symptoms, and fully recovered donors using single-nucleus multiome sequencing (snRNA-seq + snATAC-seq) after Pseudomonas aeruginosa restimulation. Systematic comparison revealed shared and specific TI features between COVID-19 and LC. Upon secondary challenge, Both TI states showed boosted type I/II IFN pathways and dampened TNF–NF-κB signalling, driven by heightened IRF and reduced C/EBP–AP-1 activity. In LC, heterologous restimulation revealed three TI subpopulations—interferon-primed, antigen-presenting, and pro-inflammatory—each defined by distinct transcriptomic and epigenomic profiles. In addition, analysis of cells in the resting state before restimulation revealed that TI memory is epigenetically imprinted through stable TF activity patterns.

Conclusions
Overall, our multi-omic data showed that LC individuals exhibit global remodelling of the chromatin accessibility landscape, indicative of the establishment of immunological memory in monocytes.

Keywords
Trained immunity; Long COVID; single multiome; Monocytes; TNF; Interferon alpha; Interferon gamma; Innate immune memory;

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TELOMERE LENGTH IN GOUT AND HYPERURICEMIA: ANALYSIS OF VARIABILITY AND ASSOCIATION WITH MONONUCLEAR CELL CYTOKINE PRODUCTION

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Objectives
Gout is an inflammatory condition with a high prevalence. It is characterized by recurrent flares driven by monosodium urate (MSU) crystals, which activate the NLRP3 inflammasome and induce the release of pro-inflammatory cytokines such as IL-1β or IL-6. Telomeres are repetitive DNA sequences at the ends of chromosomes and their main function is to prevent the loss of important genetic information during cell division. Telomere length (TL) is a key biomarker of biological aging and has been implicated in various chronic diseases, including gout. Hyperuricemia, the main risk factor for gout, is associated with increased production of reactive oxygen species (ROS), which damage DNA and accelerate telomere shortening. Moreover, the persistent inflammation observed in gout accelerates oxidative stress, a major driver of telomere attrition. Here, we investigate whether telomere length differs among gout patients, hyperuricemic controls, and normouricemic controls. We also examined whether telomere length correlates with the cytokine production capacity of mononuclear cells as higher levels of pro-inflammatory cytokines may be linked to shorter TL in immune cells.

Materials
The study was performed in the HINT study groups (patients with gout n=81, hyperuricemia n=73 and normouricemic controls n=76, Romania). Genomic DNA was isolated from whole blood and average telomere length was determined using the Absolute Human Telomere Length Quantification qPCR Assay Kit (ScienCell, CA, USA) following the supplier's instructions. Ex vivo functional assays were performed, consisting of PBMC stimulations with C16+MSU (TLR2/NLRP3 inflammasome activator) or LPS (TLR4 ligand) for 24h. Cytokines were assessed by ELISA.

Results
No significant differences in TL were observed between the groups. When stratifying the analysis by distinct age groups, variations in TL were detected, yet no overall correlation was found between TL serum urate levels, or BMI in any of the examined cohorts. Furthermore, no association was identified between TL and the pro-inflammatory cytokine IL-1b or the anti-inflammatory IL-1Ra production of peripheral blood mononuclear cells (PBMCs) in the presence of different stimuli.

Conclusions
Our findings suggest that telomere length does not significantly differ between gout patients, hyperuricemic controls, and normouricemic controls, nor does it correlate with serum urate levels, BMI, or pro-inflammatory cytokine production. Given the relatively small study group sizes assesed so far and the complexity of telomere dynamics with inter-individual variability, larger cohorts may be required to detect potential associations and fully elucidate the role of telomere length in gout and hyperuricemia inflammation.

Keywords
gout, telomere length, urate, cytokine

IL1B PROMOTER POLYMORPHISM RS16944 AND CYTOKINE PRODUCTION IN HYPERURICEMIA AND GOUT

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Objectives
Gout is a common inflammatory condition caused by the accumulation of monosodium urate (MSU) crystals in joint tissues, leading to severe joint inflammation. Interleukin-1β (IL-1β) plays a central role in mediating this inflammatory response. A specific single-nucleotide polymorphism (rs16944), located 511 base pairs upstream of the first exon of the IL1B gene, has been linked to changes in the expression of both IL-1β and the anti-inflammatory cytokine IL-37, potentially through regulation by a long noncoding RNA known as AMANZI. This study aimed to explore the relationship between rs16944 genotype, cytokine production, IL1B mRNA expression, and the frequency of gout flares.

Materials
We analyzed data from a previously characterized cohort of subjects included in the HINT project (Cluj-Napoca, Romania), that included gout patients, subjects with asymptomatic hyperuricemia and non-gout, normouricemic controls. Genomic DNA was isolated from whole blood and genotyping was conducted using Infinium™ Global Screening Array-24 v3.0 BeadChip. Cytokine production was evaluated in vitro using freshly isolated PBMCs in multiple experiment designs: 24h direct stimulation with gout-relevant stimuli (e.g. palmitate, MSU crystals), TLR ligands or microbial stimuli (n=320). 2) 24h uric acid (UA) priming followed by 24 h LPS stimulation (n=304). 3) 7-day stimulation with TLR ligands or microbial stimuli (n=236). ELISA for IL-1β, IL-1Ra, IL-6, TNF, IL-17, IFNγ was performed on cell culture supernatants. Expression of IL1B mRNA in unstimulated PBMCs and monocytes, as well as in LPS-stimulated PBMCs following UA priming was evaluated (n=47). A retrospective analysis of the frequency of flares in gout patients was conducted, with subjects (n=243) stratified in three groups: 1 flare/ year (or fewer), 2-6 flares/year and 7 or more flares/year.

Results
Following short-term stimulation, IL-1β and IL-6 production showed a trend toward increased levels in carriers of the G allele, although statistical significance was observed in only a subset of comparisons. In contrast, IL-1Ra levels displayed an inverse pattern, with consistently lower levels across all tested stimuli. This trend was also seen in IL-1β production after uric acid (UA) priming and LPS stimulation. However, baseline IL1B mRNA expression in monocytes and PBMCs was not associated with rs16944 genotype, nor was expression after UA priming and LPS stimulation. No association was found between rs16944 genotype and the frequency of gout flares.

Conclusions
This study showed a tendency towards higher proinflammatory and lower anti-inflammatory cytokine production in rs169644 G allele carriers highlighting the potential relevance of this SNP in the context of gout and hyperuricemia associated inflammation.

Keywords
gout, hyperuricemia, IL-1β, cytokines, Amanzi, rs16944

References

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LOCAL IMMUNEPATHOPHYSIOLOGY OF NON-TUBERCULOUS MYCOBACTERIAL SKIN AND SOFT TISSUE INFECTIONS

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Objectives
Non-tuberculous mycobacteria (NTM) are a group of mostly opportunistic pathogens able to cause skin- and soft tissue infections (SSTI). Risk factors include age above 50 and immunocompromised status, and the at-risk population is increasing. Treatment comprises of multidrug antibiotic regimens sometimes in combination with surgery. Despite long treatment duration with substantial associated toxicity, treatment outcomes are unsatisfactory especially in immunocompromised patients. To take informed treatment decisions and improve outcomes, a better understanding of the pathophysiology is essential. This study aims to unravel the site-of-disease immune pathophysiology through single cell RNA sequencing.

Materials
To this end, adult patients with culture-confirmed NTM-SSTI were included at Radboudumc and Erasmus MC, the Dutch referral centres for mycobacterial diseases. Both lesional and non-lesional skin biopsies were collected at the start of treatment, and a second lesional biopsy was obtained 8 weeks later. Biopsies were processed into single cell suspensions and fixated for single cell RNA sequencing.

Results
Currently, sequencing data from both before and during treatment are available for 10 NTM-SSTI patients. Analyses are in progress and focus on the differences between lesional and non-lesional skin, and the changes in lesional skin during treatment. Cell type composition and gene expression will be compared, with a focus on skin immune cell types.

Conclusions
Analyses will be finalized before the start of the symposium.

Keywords
non-tuberculous mycobacteria; skin and soft tissue infections; single cell RNA sequencing; immunepathophysiology

References
Not Applicable

MALADAPTIVE TRAINED IMMUNITY AS A THERAPEUTIC TARGET IN SARCOIDOSIS

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Objectives
Sarcoidosis is a an autoinflammatory condition marked by the formation of granuloma without evident infection. Sarcoid granuloma formation involves Th17.1 and Th1 T cells releasing inflammatory cytokines including IFN-γ in response to unknown antigens. Monocytes are recruited to tissues and respond to inflammation with epithelioid differentiation, hypertrophy and cell fusion, forming multinucleated giant cells in the center of the granuloma1. Genetic and environmental factors are known to contribute to sarcoidosis pathogenesis, but sarcoidosis etiology remains largely elusive. While immune dysregulation is well described at granulomatous lesions2, systemic dysregulation of PBMCs can be observed, marked by elevated cytokine responses (Fig. 1A), aberrant lipid metabolism3 and the capacity to form granuloma ex vivo, a phenotype that cannot be observed in healthy cells and can even be observed post cryopreservation of PBMCs4. This systemic dysregulation that is maintained ex vivo raises the hypothesis of maladaptive central trained immunity contributing to sarcoidosis pathology1.

Materials
To study implications of maladaptive central trained immunity in sarcoidosis, we analyzed PBMCs from patients suffering pulmonary sarcoidosis using single cell RNA and ATAC sequencing, combined with functional assays including LPS stimulation, ex vivo granuloma formation, CENCAT metabolic profiling and phospho-flow. For more robust biological insight and better comparability across RNA, ATAC and flow data, we focus on population-based analysis of major PBMC cell types. We then identify pathways of interest with available inhibitors and test their efficacy in the ex vivo granuloma assay.

Results
While transcriptomic analysis of major PBMC populations indicates a homogenous dysregulated state across cell types, naïve CD4 T cells stand out as most altered in chromatin accessibility. Consistent findings across transcriptome and chromatin accessibility implicate alterations in metabolism and mTOR signaling, rather than epigenetic memory, which will be followed up by CENCAT metabolic profiling and phospho-flow analysis of major PBMC populations. While mTOR is currently investigated as a therapeutic target, our study identifies PFKFB3 and HSP90 as additional putative drug targets along a similar axis.

Conclusions
While everolimus shows efficacy to inhibit ex vivo granuloma5, other drugs will be tested in this context. Our identified inhibitor targets may be implicated up- downstream of the observed mTOR-related phenotypes in granuloma formation. Further study of systemic factors or alterations in the hematopoietic origin as a cause of this systemic dysregulation is warranted.

Keywords
Sarcoidosis, single cell, transcriptomics, epigenomics, chromatin accessibility, cellular metabolism, maladaptive trained immunity

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ASSESSMENT OF TEMPORAL GLUCOSE FLUCTUATIONS AND THEIR LINK TO ATHEROSCLEROSIS AND STEM CELL FUNCTIONALITY (ATLAS TRIAL)

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Objectives
People with type 1 diabetes mellitus (T1DM) have an increased risk for cardiovascular disease (CVD) [1]. The role of inflammation in atherosclerosis and subsequent CVD has been well established [2]. We previously found increased arterial wall inflammation in T1DM but this association was independent of HbA1c values [3]. This strongly suggests that other factors besides HbA1C contribute to arterial wall inflammation in T1DM. Glycemic variability (GV) is associated with cardiovascular events and is linked to increased myelopoiesis, oxidative stress, and endothelial dysfunction [4-9]. Furthermore, previous research showed that chronic hyperglycemia induces central trained immunity in hematopoietic stem and progenitor cells (HSPC) [10]. In this cross-sectional study, we aim to investigate the effect of GV in people with T1DM on arterial wall inflammation, internal carotid artery calcification (iCAC), and central trained immunity.

Materials
Twenty people with T1DM and high GV and twenty people with T1DM and low GV will be included in this observational study. 18F-FDG-PET and full dose CT imaging will be performed in seven regions of interest. Furthermore, bone marrow aspiration will be performed to obtain HSPCs for immunophenotyping. Secondary outcomes include extensive inflammatory analyses on circulating leukocytes and detection of circulating atherogenic markers.

Results
We hypothesize that high GV increases arterial wall and systemic inflammation, and that this is partly mediated by central trained immunity in HSPCs. We expect a skew towards myeloid progenitor cells with induction of pro-inflammatory gene expression that is retained in bone-marrow derived macrophages. Furthermore, we expect that high GV is correlated with increased iCAC scores, circulating leukocytes, and cytokine production.

Conclusions
People with T1DM are at high risk for cardiovascular complications that may impact their daily life. Prevention of cardiovascular complications mainly focusses on reducing average glucose, but GV remains largely unnoticed. Several in vitro studies highly suggest that GV induces inflammation, but the link between GV and arterial inflammation in vivo is not well established. Our results could provide evidence for the clinical relevance of GV in prevention of cardiovascular complications on the long term.

Keywords
Type 1 diabetes, glycemic variability, inflammation, cardiovascular disease, nuclear imaging, trained immunity

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CHARACTERIZATION OF NEUTROPHIL FUNCTION DURING AGING

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Objectives
Aging is a natural process linked to a gradual decline of immune system function. For several immune cells, decreased cell number or function leads to a less effective immune response during aging. Neutrophils are the most abundant immune cells. They are short-lived and highly mobile immune cells with various effector functions. Due to difficulties during in vitro culturing of these cells, there is a lack of data on how neutrophils are affected with age. In this study, we aim to determine functional, transcriptomic, and epigenomic changes in neutrophils during aging.

Materials
A subset of participants from the 2024 Foresters cohort were selected to create a group of 17 young (< 30 y.o.) and 18 old (> 55 y.o.) individuals. Drawn blood was used to determine neutrophil subset frequencies and baseline activation. Isolated neutrophils were then stimulated with E. coli LPS, HK C. albicans, and PMA for 4 hours, followed by several functional assays. The neutrophil functional assaysinclude ELISA, ROS production, phagocytosis, NETosis, and Seahorse assays. DNA and RNA were also isolated to perform ATAC-seq and RNA-seq.

Results
We observed an increase in absolute neutrophil count in older individuals. Additionally, we saw an increase in activation marker CD66b in the neutrophils of older individuals. Following PMA stimulation of neutrophils for 4 hours, we observed an increased IL-8 secretion in older individuals. At baseline, ROS levels are higher in older individuals. Despite having no differences in total ROS production after stimulation, we have seen a faster onset of ROS production in response to PMA stimulation in older individuals. ATAC-sequencing results did not show any significant difference between the accessible regions of neutrophil DNA in young and old individuals. Despite this, the RNA_x0002_sequencing results show several differentially expressed genes in older individuals. Most of these upregulated genes are classified under the interferon-signaling pathway.

Conclusions
The differences observed in the neutrophil characteristic and function of older individuals show a more activated phenotype. This is exemplified by increased activation markers and ROS production at baseline of older individuals. Interestingly, the RNA-sequencing results hint at a potential role of the interferon-signaling pathway in promoting these differences.

Keywords
neutrophils, immune aging, innate immunity

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HIGHS, LOWS AND INFLAMMATORY FLOWS: THE LINK BETWEEN GLYCAEMIC VARIABILITY AND INFLAMMATION IN PEOPLE WITH DIABETES

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Objectives
Glycaemic variability (GV) has been associated to the progression of complications in both type 1 (T1D) and type 2 diabetes (T2D), but the underlying mechanism is not completely clear. We hypothesize that inflammation may be a mediating factor. The aim of this study is to determine the associations between GV and inflammation, and whether these differ between T1D and T2D.

Materials
This was a post-hoc analysis from the Hypo-METRICS study, involving 166 participants with T1D and 184 participants with insulin-treated T2D, who wore a CGM for 10 weeks. Mean glucose, standard deviation (SD), coefficient of variation (CV) and glycaemic variability percentage (GVP) were calculated from the CGM data. Circulating inflammatory proteins were measured using Olink Proteomics AB inflammation panel and compared between groups with high or low GV in both T1D and T2D. Whole blood flow cytometry was performed to determine the number of circulating immune cells (total white blood cells, neutrophils, monocytes and lymphocytes) in a subset of 31 T1D and 86 T2D participants. Spearman correlations were used for the associations with the different GV metrics.

Results
All GV metrics were higher in T1D compared to T2D ($p<0.001$). SD was 2.8 [2.3-3.3] mmol/L in T1D and 2.4 [1.9-2.9] mmol/L in T2D, CV was 32 [28-36] % in T1D and 26 [23-30] % in T2D, and GVP was 23 [18-28] % in T1D and 17 [13-21] % in T2D. Only mean glucose was comparable between both types (T1D: 8.7 [7.9-9.9] mmol/L, T2D: 8.7 [7.7-10] mmol/L). Several inflammatory proteins were higher in participants with high GV compared to low GV, but the proteins differed between T1D and T2D (e.g. TRANCE and IL-4 were higher in participants with T1D and high GV, and FGF-19 and IL-10RA in those with T2D and high GV). In T1D, mean glucose was positively correlated with the number of circulating neutrophils ($r=0.43$, $p=0.017$). Conversely, both CV ($r=-0.36$, $p=0.047$) and GVP ($r=-0.42$, $p=0.017$) were inversely correlated with the number of circulating lymphocytes in T1D. In T2D, there were no such correlations, except that GVP was inversely correlated with the number of circulating lymphocytes ($r=-0.26$, $p=0.014$).

Conclusions
GV is associated with circulating inflammatory protein levels and white blood cells, both in T1D and T2D. This association seems more pronounced in T1D, possibly because of the more profound GV in T1D. Based on these results, the link between GV and inflammation deserves further exploration.

Keywords
Diabetes, glycaemic variability, inflammation

MALADAPTIVE TRAINED IMMUNITY AS A THERAPEUTIC TARGET IN SARCOIDOSIS

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Objectives
Sarcoidosis is a an autoinflammatory condition marked by the formation of granuloma without evident infection. Sarcoid granuloma formation involves Th17.1 and Th1 T cells releasing inflammatory cytokines including IFN-γ in response to unknown antigens. Monocytes are recruited to tissues and respond to inflammation with epithelioid differentiation, hypertrophy and cell fusion, forming multinucleated giant cells in the center of the granuloma1. Genetic and environmental factors are known to contribute to sarcoidosis pathogenesis, but sarcoidosis etiology remains largely elusive. While immune dysregulation is well described at granulomatous lesions2, systemic dysregulation of PBMCs can be observed, marked by elevated cytokine responses (Fig. 1A), aberrant lipid metabolism3 and the capacity to form granuloma ex vivo, a phenotype that cannot be observed in healthy cells and can even be observed post cryopreservation of PBMCs4. This systemic dysregulation that is maintained ex vivo raises the hypothesis of maladaptive central trained immunity contributing to sarcoidosis pathology1.

Materials
To study implications of maladaptive central trained immunity in sarcoidosis, we analyzed PBMCs from patients suffering pulmonary sarcoidosis using single cell RNA and ATAC sequencing, combined with functional assays including LPS stimulation, ex vivo granuloma formation, CENCAT metabolic profiling and phospho-flow. For more robust biological insight and better comparability across RNA, ATAC and flow data, we focus on population-based analysis of major PBMC cell types. We then identify pathways of interest with available inhibitors and test their efficacy in the ex vivo granuloma assay.

Results
While transcriptomic analysis of major PBMC populations indicates a homogenous dysregulated state across cell types, naïve CD4 T cells stand out as most altered in chromatin accessibility. Consistent findings across transcriptome and chromatin accessibility implicate alterations in metabolism and mTOR signaling, rather than epigenetic memory, which will be followed up by CENCAT metabolic profiling and phospho-flow analysis of major PBMC populations. While mTOR is currently investigated as a therapeutic target, our study identifies PFKFB3 and HSP90 as additional putative drug targets along a similar axis.

Conclusions
While everolimus shows efficacy to inhibit ex vivo granuloma5, other drugs will be tested in this context. Our identified inhibitor targets may be implicated up- downstream of the observed mTOR-related phenotypes in granuloma formation. Further study of systemic factors or alterations in the hematopoietic origin as a cause of this systemic dysregulation is warranted.

Keywords
pulmonary sarcoidosis, maladaptive trained immunity, single cell, transcriptomics, chromatin accessibility, cellular metabolism

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LONG-TERM HISTONE LACTYLATION CONNECTS METABOLIC AND EPIGENETIC REWIRING IN INNATE IMMUNE MEMORY

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Objectives
To investigate whether lactate production contributes to the induction and maintenance of trained immunity and to elucidate the underlined mechanisms.

Materials
We analyzed peripheral blood mononuclear cells (PBMCs) from Bacille Calmette-Guérin (BCG)-vaccinated individuals at baseline, 2 weeks, and 3 months post-vaccination. We performed cytokine assays, lactate measurements, RNA-seq, ChIP-seq (H3K18la), and single-cell RNA-seq. In vitro human monocyte training and tolerance models were established, and pharmacological inhibitors of lactate dehydrogenase (LDH) and p300/CBP were used to modulate lactate production and histone lactylation. Additionally, host genetic polymorphisms and murine models were analyzed to assess the functional impact of lactate and histone lactylation on trained immunity.

Results
BCG vaccination enhanced glycolysis and lactate production in innate immune cells, which correlated with heightened cytokine responses to heterologous stimuli. Histone H3 lysine 18 lactylation (H3K18la) increased in trained monocytes at enhancer and promoter regions, persisted for at least 90 days in vivo, and was associated with active chromatin and enhanced gene expression. Pharmacological inhibition of LDH or p300/CBP impaired lactate production or H3K18la deposition, and trained immunity phenotypes, while host genetic variants in LDHA and EP300 modulated trained immunity responses. In mice, p300/CBP inhibition reduced BCG-induced expansion of hematopoietic progenitors, suggesting a role of epigenetic regulation in central trained immunity.

Conclusions
Lactate production and long-term H3K18la act as key molecular mediators of trained immunity by linking metabolic rewiring with durable epigenetic memory. Targeting these pathways may offer therapeutic opportunities to modulate innate immune responses in infection, vaccination, and inflammatory diseases.

Keywords
trained immunity; BCG vaccination; histone lactylation; lactate metabolism; epigenetic memory; H3K18la

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AGE-AND SEX-DEPENDENT TRANSCRIPTOMIC ALTERATIONS IN SEPSIS REVEALED BY NETWORK MODULAR ANALYSIS

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Objectives
Sepsis is a life-threatening condition resulting from a dysregulated host response to infection, leading to systemic inflammation, multi-organ failure, and high mortality. Its early diagnosis is challenging due to nonspecific symptoms, and treatment remains difficult owing to the molecular complexity and heterogeneity of the disease, which is influenced by genetic, epigenetic, age-related, and sex-specific factors. This study aimed to investigate whether baseline gene co-expression networks are preserved or disrupted in sepsis and to evaluate how age and sex modulate these transcriptional modules.

Materials
We analyzed gene expression data from healthy individuals and sepsis patients in the Romanian Functional Genomics and Severe Infections (FUSE) cohort. Weighted Gene Co-expression Network Analysis (WGCNA) was used to construct co-expression modules from healthy controls, followed by module preservation analysis in sepsis samples. Non-preserved modules were further assessed for demographic associations and biological relevance using upstream regulator prediction, prognostic modeling, and pathway enrichment analysis.

Results
We identified fifteen co-expression modules in healthy individuals, of which four were disrupted in sepsis. Notably, one disrupted module ("Green") was significantly associated with sex and with individuals younger to middle-aged range. We identified 13 age-associated and 20 sex-associated hub genes with high predictive accuracy (AUC ≈ 0.98), confirmed by DeLong's test. Importantly, 23% of genes in the Green module were not detected as significant by conventional differential expression analysis after multiple testing correction, reinforcing the importance of a modular approach. Regulatory analysis identified key DNA motifs and transcription factors potentially driving the expression of hub genes. Functional enrichment implicated pathways involved in cellular senescence, chromatin remodeling, stem cell maintenance, immune regulation, and inflammation which are hallmarks of both aging and sepsis.

Conclusions
Sepsis disrupts baseline transcriptional networks in a manner strongly influenced by age and sex. Integrating co-expression analysis with demographic stratification enhances our understanding of sepsis biology. These findings underscore the importance of incorporating age- and sex-specific considerations into precision diagnostics and personalized treatment strategies for sepsis.

Keywords
Sepsis; Gene co-expression networks; WGCNA; Age; Sex;Immune regulation

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MOLECULAR PATHWAYS AFFECTED BY CLARITHROMYCIN TREATMENT IN COMMUNITY-ACQUIRED PNEUMONIA: INSIGHTS FROM THE ACCESS TRIAL

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Objectives
The double-blind, randomized, placebo-controlled ACCESS trial in hospitalized patients with community-acquired pneumonia (CAP) showed that adding clarithromycin to standard-of-care (SoC) therapy accelerated symptom resolution and organ function recovery within the first 72 hours and reduced progression to organ dysfunction and secondary sepsis [1]. It is yet to be seen whether clarithromycin has an immuno-modulatory activity through "omics" approaches.

Materials
This sub-study involved peripheral blood sampling at baseline (prior to treatment) and 72 hours after treatment (day 4). mRNA sequencing and protein quantification using the Olink Target 96 inflammation panel were conducted at both time points. The gene and protein expression changes were assessed using DESeq2 and OlinkAnalyze R packages, respectively. Differentially expressed genes (DEGs) and proteins were identified between treatment arms and over time within arms. Gene Ontology (GO) and Reactome pathway analyses were applied to significant DEGs (FDR-adjusted $P < 0.05$).

Results
Transcriptomic analysis was conducted on 172 samples from 86 patients, while proteomic profiling was performed on 394 samples from 197 patients. A total of 74 patients had data available for both -omics layers. At baseline, no significant differences in gene or protein expression were observed between the groups. On day 4, 13 downregulated genes were identified in the clarithromycin group, predominantly associated with interferon signaling pathways. Longitudinal analysis within treatment arms indicated that uniquely upregulated genes in the clarithromycin group were enriched for T-cell activation and positive regulation of cytokine production. Notably, interferon-signaling pathways (alpha, beta, and gamma) were downregulated over time in both groups. At the protein level, longitudinal analysis revealed that IFN-gamma was significantly downregulated in both placebo and clarithromycin arms. In contrast, IL6, a pro-inflammatory cytokine implicated in CAP severity, was selectively downregulated only in the clarithromycin group. Several other inflammation-related markers, including CXCL9, CXCL10, and CCL19, were uniquely suppressed in the clarithromycin group, underscoring its broader immunomodulatory impact.

Conclusions

Treatment with clarithromycin in hospitalized patients with CAP exerts distinct immuno-modulatory effects, characterized by suppression of pro-inflammatory cytokines (e.g., IL-6, CXCL9, CXCL10) and interferon signaling, alongside enhanced expression of genes linked to T-cell activation and cytokine regulation. These molecular shifts may underlie the clinical benefits observed in the ACCESS trial and support the adjunctive use of clarithromycin as host-directed therapy in severe CAP.

Keywords

community-acquired pneumonia, clarithromycin, sepsis, transcriptomics, proteomics

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EXPLORING THE EFFECT OF IMPAIRED GLUCOSE HOMEOSTASIS ON IMMUNE CELL METABOLISM

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Objectives
Immune cell function is tightly connected to cellular metabolism, which may be altered in people with impaired glucose homeostasis (IGH). In Maternally Inherited Diabetes and Deafness (MIDD), a mitochondrial disease, oxidative phosphorylation (OXPHOS) is known to be impaired. On the other hand, people with type 1 diabetes mellitus (T1D) are often in a state of chronic low-grade inflammation, which is associated with increased dependence on glycolysis. In this study, we used Cellular Energetics through Non-Canonical Amino acid Tagging (CENCAT) to gain a better understanding of immune cell metabolism in people with IGH in relation to the healthy population.

Materials
In this study we performed CENCAT on peripheral blood mononuclear cells (PBMCs) from 11 persons with MIDD, 14 with T1D and 15 healthy controls (HC). CENCAT is a flow cytometry-based method which measures cellular protein translation as a proxy for metabolic activity, allowing for determination of relative cellular dependence on mitochondrial and glucose metabolism after application of small-molecule metabolic inhibitors.

Results
The total population was 50% female, aged 48 [31-57] years and had a BMI of 24.3 [22.0-26.3] kg/m2. HbA1c (mmol/mol) was significantly higher for both MIDD (53 [51-57]) and T1D (56 [51-67]) compared to HC (34 [32-37], P<0.05). Mitochondrial dependence of classical monocytes was lower for MIDD compared to HC (27.9 [20.8-41.0]% vs 47.1 [39.7-61.7]%, P<0.05), with that of T1D being in the middle (37.9 [28.6-48.1]%). Mitochondrial dependence was also lower for early natural killer cells in MIDD compared to HC (69.5 [62.6-73.0]% vs 78.0 [68.2-84.6]%, P<0.05). For T1D, HbA1c was negatively correlated with mitochondrial dependence for naive CD4 T cells (r=-0.65, P<0.05) and naive CD8 T cells (r=-0.64, P<0.05). Glucose dependence was higher in MIDD compared to HC for B cells (66.3 [62.8-72.3]% vs 57.4 [52.1-63.3]%, P<0.05) and effector CD8 T cells (58.6 [44.5-61.8]% vs 46.0 [36.5-53.4]%, P<0.05), which was not found for T1D. Furthermore, in people with MIDD there was a negative correlation between HbA1c and glucose dependence in effector CD8 T cells for MIDD (r=-0.64, P<0.05).

Conclusions
People with MIDD had lower mitochondrial dependence for multiple myeloid PBMC subsets and higher glucose dependence for other subsets compared to HC. These effects were not clearly seen in people with T1D, but the correlations with HbA1c may indicate an immunomodulating role of glucose control that requires further study.

Keywords
immunometabolism; diabetes; CENCAT

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IDENTIFICATION OF NON-CODING RNAS CONTROLLING GOUT-RELEVANT GENES

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Objectives
Gout is characterized by flares caused by monosodium urate (MSU) crystal deposition and subsequent activation of innate immune cells. A recent genome-wide association study (GWAS) identified many gout-associated variants that may affect genes related to this immune response. It is important to translate these genetic associations into mechanistic and molecular insights for new druggable targets to prevent gout flares. Hereby, we focus on non-coding RNAs (ncRNAs) controlling the transcription levels of putative gout flare-related genes that are not associated with hyperuricemia. This includes the previously unknown CSF1-CSF1R axis in gout.
In this study, we aimed to investigate a potential immune gene-priming long non-coding RNA (IPL) and enhancer RNA (eRNA) of colony-stimulating factor 1 (CSF1) and assessed its role in gouty arthritis.

Materials
Characterization was done on the transcription levels (IPL, eRNA, and mRNA) in a control and stimulated setup to validate the presence of these RNAs and their effects on CSF1 concentrations. Initial experiments were done in THP-1 cells to establish the technology needed for the extraordinarily low expressed RNAs. Based on the GWAS hits, we focused to validate the IPL, eRNA, and CSF1-mRNA in qPCR and dPCR/ddPCR experiments. Furthermore, the Olink proximity extension assay was used to determine CSF1 plasma concentrations in gout patients within a flare or the inter-critical phase.

Results
In order to study specifically CSF1 as well as its potential regulatory ncRNAs, we designed an experimental setup upregulating CSF1 expression. Initial experiments were carried out with 4 to 96 hours PMA-stimulated THP-1 cells, since they had higher availability and higher RNA concentrations. This allowed us to verify upregulation of CSF1 and CSF1R by PMA and the link between the eRNA and IPL transcription. Whereby CSF1 and CSF1R show a continuous increase over the time course, the ncRNAs suggest an initial peak after 4h stimulation, thus suggesting a different order of activation by PMA. Future experiments will be focussed on primary human monocytes of gout patients, resembling in vivo settings.

Conclusions
This study improves the understanding of the transition from hyperuricemia to gout by focusing on gout-associated genes that do not appear to play a role in hyperuricemia.
Furthermore, unravelling the molecular pathways will improve therapeutical interventions by providing novel targets for gout.

Keywords
gout, ncRNA, lncRNA, eRNA, CSF1, CSF1R, gene

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Gazova et al., 2020, Fanucchi et al., 2019, Fok et al., 2024

HIGH-RESOLUTION NANOLIQUID CHROMATOGRAPHY–MASS SPECTROMETRY OF CIRCULATING IGA1 GLYCOSYLATION PROVIDES NOVEL INSIGHT IN THE PATHOGENESIS OF IGA NEPHROPATHY

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Objectives

It is believed that abnormal O-glycosylation of the IgA1 HYT peptide in the hinge region, also known as galactose-deficient IgA1 (gd-IgA1), is a prerequisite and "Hit 1" for the development of IgA nephropathy (IgAN). However, limited information is available regarding glycosylation of immunoglobulins in IgAN patients. Here we present the first high-resolution molecular characterization of protein-specific O- and N-glycosylation of circulating immunoglobulins in IgAN patients compared to age- and kidney function-matched controls.

Materials

We used LC-MS for the glycopeptide analysis of circulating IgA of 41 IgAN patients that were not treated with corticosteroids or other immunosuppressants, and 41 age- and eGFR-matched controls. Gd-IgA1 was measured in plasma with the monoclonal KM-55 antibody using a commercially available ELISA assay (MBS109533).

Results

Using LC-MS analysis, we identified 92 O- and N-glycopeptides and quantified 30 glycosylation traits. We found that galactose on the IgA1 HYT peptide was lower in IgAN patients compared to controls. Interestingly, the galactose content of 40 (98%) IgAN patients fell within the range observed in controls. ELISA-measured gd-IgA1 levels were, on average, higher in IgAN patients than in controls, with 33 (80%) IgAN patients having values within the control range. Gd-IgA1 correlated positively with terminal GalNAc on the HYT peptide, and negatively with galactose on the HYT peptide, indicating that terminal GalNAc is the epitope for the ELISA-measured gd-IgA1. Interestingly, one N-glycan, a LAGY peptide with low sulfation, was significantly higher in IgAN patients and was also associated with proteinuria. Multiple N-glycosylation traits, mainly sialylation of the IgA1 heavy chain peptides, correlated significantly with kidney function.

Conclusions

We confirm that terminal GalNAc on the IgA1 HYT peptide is the epitope for the ELISA-measured gd-IgA1. Interestingly, the galactose level on the HYT peptide for most IgAN patients falls within the range observed in their kidney function-matched controls, suggesting that it could be considered a naturally occurring epitope rather than the result of aberrant glycosylation. Furthermore, N-glycosylation traits on the IgA heavy chain are associated with disease severity. This provides new insight in IgAN pathophysiology, since heavy chain N-glycosylation changes are known to be associated with immune cell activation.

Keywords IgA nephropathy, glycosylation, mass spectrometry, KM55 antibody

IMMUNE MODULATION BY LENVATINIB IN PATIENTS WITH NON-MEDULLARY THYROID CANCER

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Objectives
Lenvatinib is an approved tyrosine kinase inhibitor for the treatment of advanced thyroid cancer (TC), but patients inevitably present with progressive disease and complete remissions are rare. Previous studies have indicated that lenvatinib influences the immune system, specifically on cells of the myeloid lineage. However, the immunomodulatory effects of lenvatinib have not yet been studied in patients with advanced TC.

Materials
Peripheral blood was collected from lenvatinib-treated patients to establish a cross-sectional and a longitudinal cohort. Systemic immune profiling was performed by cell counts and proteomic analysis in EDTA blood. Peripheral blood mononuclear cells (PBMCs) and monocytes were isolated for ex vivo stimulation assays to assess cytokine production capacity. To further investigate functional effects, healthy donor monocytes were used to evaluate metabolic activity, reactive oxygen species (ROS) production, and phagocytosis. Lastly, we performed proteomics and surface marker analyses on the TPC-1 thyroid cancer cell line to assess tumor-intrinsic responses to lenvatinib.

Results
In circulation, lenvatinib treatment resulted in an increase in lymphocytes and decrease in neutrophils. In both patient cohorts, lenvatinib treatment highly modulated the inflammatory proteome indicated by elevated levels of VEGFA, CCL11, MMP-10, and TRAIL. Ex vivo lenvatinib treatment highly attenuated production capacity of IL-1β, IL-6, IL-8, and IL-10. In the cross-sectional cohort, lenvatinib treatment was associated with enhanced production capacity of IL-1Ra, TNF-α, and IFN-γ. Healthy donor monocytes demonstrated a reduced glycolytic capacity and an increase in ROS production. Lenvatinib treatment altered the secretome in TPC-1 tumor cells, and elevated surface marker expression of major histocompatibility complex (MHC) class I, programmed death ligand 1 (PD-L1) and CD40.

Conclusions
This multi-compartment study demonstrates that lenvatinib induces broad immunomodulatory effects in patients with advanced thyroid cancer, including shifts in immune cell populations, changes in the circulatory proteome, and reprogramming of cytokine responses in monocytes and PBMCs. Lenvatinib also impacts immune cell metabolism and tumor cell phenotype. These findings provide insight into the immunological activity of lenvatinib and highlight opportunities for biomarker discovery and combination strategies.

Keywords
Non-medullary thyroid carcinoma, lenvatinib, immunomodulation, circulatory biomarkers, macrophages, monocytes.

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ENHANCING TUBERCULOSIS VACCINE EFFICACY WITH A HETEROLOGOUS MRNA-CHADOX1 PRIME-PULL STRATEGY TARGETING LUNG-RESIDENT MEMORY T CELLS

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Objectives
Tuberculosis (TB) remains a major global health threat, necessitating the development of vaccines that are more effective than Bacillus Calmette-Guérin (BCG). Building on previous findings where homologous prime-boost vaccination with mRNA encoding the promising mycobacterial antigen PPE15 (mRNA.PPE15) failed to improve BCG efficacy, we investigated a heterologous strategy combining intramuscular mRNA.PPE15 priming with intranasal boosting using a chimpanzee adenovirus vector (ChAdOx1.PPE15), aiming to enrich lung-resident memory T cells (TRM). We showed that this BCGmRNA.PPE15-ChAdOx1.PPE15 regime elicited robust systemic and pulmonary immune responses and improved the efficacy of BCG by significantly reducing Mycobacterium tuberculosis (M.tb) bacterial loads in the lungs and spleens of vaccinated mice. Using intravascular staining and PPE15-specific tetramer assays, we observed the localisation of PPE15-specific TRM cells within the lung parenchyma, a phenotype previously associated with superior protective immunity against TB. These findings demonstrate that a prime-pull strategy can direct antigen-specific cellular responses to the lungs, highlighting the importance of mucosal targeting and vaccine administration sequence in TB vaccine design. This heterologous prime-pull vaccination approach is promising for next-generation TB vaccines and warrants further investigation in clinical settings.

RELIABILITY OF INFRARED THERMOGRAPHY FOR THE ASSESSMENT OF DIABETIC FOOT COMPLICATIONS

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Objectives

Background: Infrared thermography cameras have the potential to improve diabetic foot care, which can map the temperature over the whole foot. Left-right comparison of the foot temperature are made, where large differences can indicate local inflammation. This information can be used to monitor Charcot arthropathy and prevent and monitor diabetic foot ulcers. Currently, the IRT methodology for foot measurements in literature is not consistent. Examples of inconsistencies include the use of different views, patient position, the room temperature, acclimatization time and the method of body coverage during the measurements. Therefore, before clinical applicability is further investigated, the aim of this study is to investigate potential influential factors for foot IRT measurement in order to establish a reliable methodology.

Materials

Methods: Healthy participants will be included in the study (N=20). Main focus points include the minimal acclimatization time, the influence of room temperature and comparison to alternative measurement methods. Medial, lateral, dorsal and plantar views are included to capture all relevant areas of the foot. The measurement reliability will be expressed by both the absolute and asymmetric temperature difference between subsequent measurements. In developing the measurement system, the goal is to strike a balance between minimizing noise contributors while maintaining a setup and measurement procedure that is workable for potential future clinical use.

Conclusions

Discussion: A robust and reliable measurement method is crucial when trying to introduce a new diagnostic tool to clinical care. Therefore, the impact of several confounders (e.g. acclimatization time and room temperature) will be analyzed, which in previous studies are often unreported or vary greatly. Both the absolute temperature and the asymmetrical temperature difference between the right and left foot (ΔT) will be investigated. It is expected that the ΔT will be less sensitive to room temperature changes compared to the absolute temperature difference.

Keywords

Infrared thermography, infrared thermal imaging, diabetic foot complications, validation, measurement reliability, inflammation

LONGITUDINAL PROTEOMICS IN ERYTHEMA MIGRANS PATIENTS

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Objectives
Lyme disease, caused by the spirochete Borrelia burgdorferi, often presents with erythema migrans (EM), a characteristic skin lesion. Early immunological events during EM are poorly characterized at the proteomic level, and understanding these could provide insights into disease pathogenesis and recovery. This study aimed to longitudinally profile serum proteomic changes in EM patients and compare them to healthy controls to better understand the early immune response against the bacteria.

Materials
We enrolled 10 patients diagnosed with EM and 10 healthy controls in the study. Serum samples from EM patients and healthy controls were collected at four timepoints: baseline (diagnosis), 2 weeks, 6 weeks and 3 months post diagnosis. Proteomic profiling was performed using proximity extension assay technology (Olink Explore 3072/384).

Results
Unadjusted analyses at baseline revealed trends toward increased expression of several immune mediators in EM patients, including proteins relevant to Th17 cell responses. Upregulated proteins in EM patients were IL17C and IL22RA1 e.g., both closely associated with Th17 responses. Other upregulated proteins of interest were: ARNT, IL-1RL2, JUN and REG4, while TNFSF10, CXCL9, CXCL3, IL1RN, CXCL10 and IL-22RA1 and TNF were downregulated. Longitudinal analysis within the EM group showed dynamic changes in IL17F, IL24, IL20, and IL20RA over the course of infection and recovery, indicating evolving Th17 pathway activity. These findings suggest that Th17-related cytokines are modulated during early Lyme disease and convalescence. However, after correction for multiple comparisons, none of the observed protein changes reached statistical significance, reflecting the limited sample size and high dimensionality of the dataset.

Conclusions
Our longitudinal proteomic analysis of EM patients demonstrates dynamic regulation of Th17-associated cytokines, including IL17C, IL17F, IL22RA1, and IL24, during the course of Lyme disease. While statistical significance was not achieved after adjustment, the observed trends support a role for Th17 responses in the immunopathogenesis and resolution of early Lyme disease. These findings warrant validation in larger cohorts and suggest that Th17 pathways may be promising targets for future biomarker and therapeutic studies in Lyme disease.

Keywords
Borrelia burgdroferi, erythema migrans, proteomics, Th-17 response

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VARIATION IN PBMC CYTOKINE RESPONSE: HOW MUCH CAN WE EXPLAIN?

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Objectives
The extent of immune response to various molecular signatures likely influences disease susceptibility. We observe a marked variability in cytokine production during ex vivo stimulation assays. Therefore, we aimed to quantify the contribution of genetic and non-genetic factors to the variability in peripheral blood mononuclear cell (PBMC) cytokine response to various stimuli seen between individuals.

Materials
We used data from the 200FG cohort, including a total of 547 healthy participants from the Netherlands. Each year, PBMCs from ~200 of these participants are stimulated with a variety of stimuli for 24h or 7 days. Cytokines are measured in the supernatants using ELISA. We also have whole genome genotyping data on 431 of the 547 participants. For this study, data from the 2017, 2018, and 2023 cohorts were included (N = 152, 201, and 194 respectively). The 2017 cohort included stimulation with C16 fatty acid with or without monosodium urate (MSU) crystals. The 2018 cohort included stimulation with lipopolysaccharide (LPS), basic calcium phosphate (BCP) crystals, and the combination. The 2023 cohort included stimulation with LPS, calcium pyrophosphate (mCPPD) crystals, and the combination. All stimulants were prepared in a single batch, all stimulations were 24 hours long, and in all years IL-1β, IL-6, and IL-1RA were measured in the supernatant. For each cytokine/stimulus pair, we first performed a GWAS, then we measured the percentage of variance explained (R2) by age, gender, PBMC lymphocyte:monocyte ratio, PBMC neutrophil percentage, date of stimulation, and polygenic risk scores comprised of all independent variants associated at $P < 10^{-5}$ after adjusting for all other variables listed.

Results
We found substantial variability in cytokine response across all stimuli, and that this variance was explained by a variety of factors. Notably, PBMC lymphocyte:monocyte ratio explained up to 42% (median 5%) of variance in cytokine response, date of stimulation explained up to 63% (median 11%), and the polygenic risk scores explained up to 64% (median 16%) of variance.

Conclusions
This study highlights the importance of genetics, PBMC cell composition, and stimulation batches in determining the extent to which an individual responds to a stimulus. It suggests that individuals who are predisposed to have higher cell counts of monocytes relative to lymphocytes or higher responsiveness to stimuli could have elevated risk for developing diseases such as gout, osteoarthritis, or CPPD disease.

Keywords Genetics, Cytokine, Immunology
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OVERCOMING IMMUNE SUPPRESSION IN OVARIAN CANCER BY REPROGRAMMING CENTRAL INNATE IMMUNE MEMORY

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Objectives
Ovarian cancer (OC) remains one of the deadliest gynecologic malignancies, largely due to late-stage diagnosis and inevitable disease recurrence despite aggressive first-line therapies. The five-year survival rate has stagnated at ~30% over the past two decades, highlighting the urgent need for novel therapeutic strategies. Emerging evidence suggests that dysregulated myeloid cell function and impaired innate immune memory may contribute to immune evasion in OC.

Materials
We are currently profiling immune cells and their progenitors in patients with advanced OC undergoing primary or interval debulking surgery, alongside matched healthy controls. Cells from bone marrow, peripheral blood, spleen, intraperitoneal fluid, and tumor tissue will be analyzed with flow cytometry, RNA sequencing, and ATAC-seq. Functional assessment of trained immunity was conducted using ex vivo stimulation assays on peripheral blood and bone marrow-resident mononuclear cells as well as bone marrow progenitors.
Parallel studies in murine models of OC enabled comparison of systemic and local immunophenotypes and allows for the evaluation of trained immunity–modulating compounds in vivo.

Results
Stem and immune cells from OC patients show a distinct immunosuppressed phenotype, which can be overturned through the induction of trained immunity. A similar phenotype is observed in OC-bearing mice when focusing on mature immune cells and the myeloid progenitors.
This indicates that the targeting of innate immune memory can be a compelling novel therapeutic strategy to overcome immune suppression in OC.

Conclusions
These findings uncover a central role for defective innate immune memory in ovarian cancer–associated immunosuppression. Therapeutically reprogramming myeloid progenitors to restore trained immunity may represent a promising immunotherapeutic approach for overcoming resistance in OC.

Keywords
Ovarian cancer ; Innate immune memory ; Myeloid progenitors ; Immunosuppression

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SOLVE-IEI: SOLVING ENIGMAS OF UNDIAGNOSED INBORN ERRORS OF IMMUNITY USING LATEST GENOMIC TECHNOLOGIES

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Objectives
Inborn Errors of Immunity (IEI) include over 500 monogenic disorders affecting immune system, encompassing primary immunodeficiencies, autoinflammatory diseases, and autoimmune conditions. Despite advances in next-generation sequencing (NGS), many individuals with strong clinical suspicion of IEI remain without a definitive molecular diagnosis after initial exome or genome sequencing. The Solve-IEI project seeks to bridge this diagnostic gap by applying advanced genomic and multi-omic approaches to uncover the genetic causes in these unresolved cases.

Materials
First, we are systematically re-analyzing existing non-contributory exome and genome sequencing data from a large local series of approximately 400 individuals with suspected IEI, using updated bioinformatics re-annotation pipeline.
Second, we are implementing long-read HiFi genome sequencing in a selected subset of individuals with a strong suspicion of monogenic IEI but inconclusive clinical NGS. Selection is based on strict criteria including early-onset symptoms, severe phenotypes, or syndromic features (e.g. neurodevelopmental disorder, malformation syndrome, abnormal craniofacial features etc.). Samples for this work are sourced through an ongoing European collaboration involving centers in the Netherlands, France, Austria, and the United Kingdom. Additionally, targeted long-read HiFi sequencing is performed in individuals with phenotypes strongly suggestive of specific genes, such as MEFV in Familial Mediterranean Fever (FMF), when no or only a single pathogenic variant has been identified by standard of care approach.
The third research axis employs a multi-omics approach for the functional characterization of known IEI-causing genes. Our strategy is to profile and compare dynamic changes in gene expression using Iso-Seq and long-read derived methylomics data in PBMCs before and after in vitro stimulation with tailored pathogens. Analyzing these differential changes between individuals with well-established IEI (e.g. STAT1-related chronic mucocutaneous candidiasis) and healthy controls is anticipated to define a “disease-specific molecular signature” of the immune response, an innovative concept paving the way for precision immunology.

Results
The re-analysis of NGS data has already identified novel candidate variants in genes not previously associated with IEI, such as NFKBIZ and NFATC4, for which a matchmaking process is underway to find additional individuals and reinforce evidence of causality. In parallel, targeted long-read analysis of MEFV has successfully resolved 4 out of 28 individuals analyzed to date.

Conclusions
By integrating comprehensive clinical phenotyping with state-of-the-art genomic technologies, the Solve-IEI project aims to improve the diagnostic yield for individuals with genetically unsolved IEI. This holistic strategy will not only provide crucial answers for affected individuals and their families but also enhance our understanding of the molecular mechanisms underlying immune function and dysfunction.

Keywords
Genomic technologies, long-read sequencing, inborn errors of immunity, genetic diagnostics.

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HEAL-SEPSIS: A SYSTEMS IMMUNOLOGY APPROACH TO CHARACTERIZE THE IMMUNE RESPONSE IN SEPSIS AND ITS LONG-TERM COMPLICATIONS

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Objectives

Background: Sepsis is a life-threatening organ failure syndrome caused by a dysregulated host response to infection. Despite advances in supportive care and antimicrobial therapy, mortality remains high due to our inability to rebalance the host immune response. Sepsis survivors are also at increased risk of long-term complications, such as recurrent infections and cardiovascular comorbidities. Current immunotherapy strategies fail to account for the highly heterogeneous immunopathological mechanisms underlying sepsis. Some patients experience hyperinflammation, others immune paralysis, with these states often coexisting or shifting over time. Moreover, different immunological mechanisms may drive each of these broad patterns of immune dysregulation. To enable the development of targeted immunotherapies, patients need to be classified according to shared immune response profiles—referred to as immunotypes—and clinically applicable biomarkers are needed to differentiate between them. In addition, the longterm morbidity and mortality following sepsis, potentially driven by lasting epigenetic changes, are poorly understood. A deeper understanding of immunotypes and their impact on both acute and long-term outcomes is essential to improve patient stratification and guide future precision medicine approaches in sepsis. Objective: This study aims to characterize sepsis immunotypes linked to short- and long-term outcomes and identify novel biomarkers and therapeutic targets

Materials

Methods: We are conducting a multi-center, prospective observational cohort study involving 400 adult patients meeting Sepsis-3 criteria who are admitted to the Intensive Care Unit. Participants will be retrospectively stratified into subgroups based on the site of infection. Blood samples will be obtained at day 0 (within 48 hours of ICU admission), day 3 (defined as 3 days after day 0), at hospital discharge and at 3 and 12 months post-discharge. Multi-omics data, including transcriptomics, proteomics, metabolomics, and epigenetics will be integrated with immunological data to characterize immunotypes. To link the immunotypes to short- and long-term outcomes, we will gather clinical parameters such as survival, hospital readmission, and infectious and cardiovascular events up to 12 months after hospital discharge. By integrating clinical outcomes with multi-omics data, we aim to define clinically relevant sepsis immunotypes and identify diagnostic markers and molecular pathways associated with each immunotype.

Keywords

Sepsis, immunotypes, short- and long term outcomes, observational.

COLCHICINE MODULATES IMMUNE-CELL ACTIVATION AND TARGETS CHEMOKINE PRODUCTION

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Objectives
Colchicine is an ancient anti-inflammatory drug that exerts its effects by disrupting intracellular microtubule dynamics in immune cells. It is well known for inhibiting inflammasome assembly and subsequent IL-1β secretion, making it highly effective in treating auto-inflammatory conditions such as gout (1). While its systemic immunosuppressive effects are well established, the specific effects of colchicine on individual innate immune cell types, particularly monocytes, remain poorly understood (2). The objective of this study is to assess the in vitro effects of colchicine on primary human peripheral immune cells, in the context of a gout-flare, as well as its effects on macrophage differentiation.

Materials
PBMCs were isolated from healthy volunteers using Ficoll-Paque density gradient centrifugation. Monocytes were subsequently separated through hyper-osmotic density gradient centrifugation. The isolated cells were cultured with a 1-hour pre-incubation of colchicine (50nM-500nM), followed by a 24-hour stimulation with monosodium urate (MSU) crystals and lipopolysaccharide (LPS). Supernatants were collected for cytotoxicity assessment and protein quantification, while the cells were processed for RNA isolation, analyzed by Seahorse metabolic assay and measured for ROS and phagocytosis capacity. In parallel, monocytes were differentiated into macrophages by culturing them with either GM-CSF or M-CSF for six days in the presence or absence of colchicine (1nM-50nM). Macrophage differentiation and marker expression were assessed using flow cytometry.

Results
Colchicine treatment of stimulated primary monocytes inhibits the transcription and the extracellular secretion of pro-inflammatory cytokines (IL-1β, IL-6, TNF). This effect is annulled when the same conditions are applied to PBMCs, where colchicine treatment increases IL-1β secretion in the extracellular space. Metabolically, colchicine impacts both oxygen consumption and cellular glycolysis. Colchicine also reduces monocyte ROS production and phagocytosis. The strongest effect of colchicine on PBMCs and monocytes is the inhibition of RNA transcription and protein production of monocyte chemoattractant proteins, including MCP-1, MCP-2, MCP-3, CXCL9 and CXCL10. Finally, colchicine affects monocyte to macrophage differentiation, in both GM-CSF and M-CSF differentiated cells, by inducing a higher expression of CD86 and a lower expression of CD206, CD163 and CD14.

Conclusions
In this study, we described a range of colchicine's effects on primary human immune cells, by using an invitro gout-flare model. Beyond preventing inflammasome activation, colchicine also inhibits monocyte recruitment by suppressing chemokine production. These findings suggest a broader therapeutic potential for colchicine, in addition to its current uses.

Keywords
Monocytes, PBMCs, Colchicine, Inflammation, Chemokines, Cytokines

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EPIGENETIC LOCKDOWN OF AP-1 SITES IN MONOCYTES DISRUPTS IMMUNE FUNCTION IN LONG COVID

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Objectives
The COVID-19 pandemic inflicted devastating global morbidity and mortality through acute infection, but its legacy extends far beyond the initial viral clearance. An estimated 6-10% of those infected went on to develop persistent symptoms, ranging from debilitating fatigue to cognitive impairment, collectively known as long COVID. Despite growing recognition of this syndrome, the underlying cellular and molecular immune mechanisms driving long-term dysfunction remain poorly understood.

Materials
Participants with prior COVID-19 infection were recruited from the COVAS and DECI-LOCO cohorts and stratified into three groups (n = 20 per group) based on the severity of long COVID symptoms: none, mild, or severe. Plasma samples were analyzed using the Olink® Target 96 Inflammation panel to profile circulating inflammatory proteins. Monocytes were isolated from peripheral blood, subjected to ATAC-sequencing to assess chromatin accessibility, and stimulated ex vivo with LPS to evaluate cytokine secretion, specifically TNFα, IL-6, and IL-1β.

Results
Olink® proteomics analysis of plasma revealed elevated levels of inflammatory proteins, primarily chemokines, in individuals with severe long COVID. These participants also exhibited altered immune cell composition, with decreased monocyte and increased lymphocyte counts. Functionally, monocytes from the severe group displayed marked immune hypo-responsiveness, producing significantly lower levels of TNFα and IL-1β following ex vivo LPS stimulation. ATAC-sequencing of these monocytes uncovered widespread epigenetic remodeling. Notably, there was a significant increase in regions of chromatin inaccessibility, the extent of which correlated with long COVID symptom severity. Motif enrichment analysis revealed that these closed regions were strongly, and nearly exclusively, depleted of AP-1 binding motifs, suggesting a targeted loss of AP-1–mediated transcriptional control. In contrast, regions showing increased accessibility were enriched for motifs of the ATF3 transcription factor family, demarkating immune suppression and physiological stress. Pathway analysis further implicated enrichment of glucocorticoid- and corticosteroid-responsive genes in monocytes from severe long COVID patients, pointing to a possible stress- or hormone-mediated axis contributing to the observed immune dysfunction. EBV and CMV viral genome reads were detected to a higher degree in monocytes of severe long-COVID participants.

Conclusions
We have oberved a clear pattern of immune dysregulation in the blood of severe long-COVID. This immune dysregulation is upheld by an increase in plasma inflammatory proteins, an increase in the

lymphocyte to monocytes ratio, where monocytes showed changes to epigenetic accessibility of AP-1 transcription factor motifs which can be tied to clear deficits in monocyte immune function in severe long-COVID.

Keywords
Long-COVID, Monocyte, Immune dysregulation, ATAC-sequencing, Epigenetic accessibility, AP-1, ATF3, Viral persistence

SERUM METABOLOMICS REVEALS DYSLIPIDAEMIA IN GOUT AND HYPERURICEMIA: EXPLORING INFLAMMATORY LINKS THROUGH INTEGRATIVE MULTI-OMICS

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Objectives
Gout and asymptomatic hyperuricemia (AH) are characterized by both metabolic dysregulation (1) and systemic inflammation (2), yet the interplay between lipid alterations and inflammatory responses remains insufficiently understood. Nuclear magnetic resonance (NMR)-based metabolomics enables the characterization of metabolite and lipid species, providing insights into metabolic disturbances associated with these conditions. While dyslipidaemia, including elevated very-low-density lipoprotein (VLDL), has been previously reported in gout and AH (3), its relationship with inflammatory pathways remains unclear.
This study aims to: (i) identify metabolomic and lipidomic alterations distinguishing gout and AH from normouricemic controls; (ii) investigate correlations between these metabolic shifts and in vivo inflammatory responses; and (iii) explore potential mechanistic links in vitro through peripheral blood mononuclear cell (PBMC) stimulations.

Materials
Serum samples from patients with gout, AH, and normouricemic controls were analyzed using an NMR-targeted metabolomics approach to profile lipid and metabolic alterations. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were employed to identify key metabolites differentiating groups, while correlation analyses with inflammatory serum proteins provided insights into lipid-associated immune activation.

Results
Our data reveals a distinct lipidomic profile in gout and AH, characterized by alterations in multiple lipid species, including VLDL-related fractions and other lipoprotein-associated metabolites. These metabolic changes show correlations with inflammatory serum proteins, suggesting a potential interaction between dyslipidemia and immune activation.

Conclusions
This study integrates targeted metabolomics and proteomics to examine metabolic-inflammatory interactions in gout and AH. By integrating NMR-based metabolic profiling with inflammatory protein signatures in vivo and further functional immune responses in vitro, we demonstrate that dyslipidemia may play a role in immune activation, potentially informing metabolic and immunomodulatory therapeutic strategies.
These investigations aim to determine whether targeting lipid alterations could offer novel avenues for reducing inflammation in hyperuricemic conditions.

Keywords
Hyperuricemia; Gout; Inflammation; Metabolomics; Dyslipidemia.

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BIOMARKER FOUND IN PLASMA HAS POTENTIAL TO DIFFERENTIATE BETWEEN GOUT AND CPPD DISEASE

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Objectives
Clinical differentiation between gout and calcium pyrophosphate deposition (CPPD) disease is challenging, as during acute flares, patients with either condition typically exhibit symptoms such as pain, swelling, and reduced range of motion in the affect joint. A thorough understanding of the unique characteristics of each condition is essential for accurate diagnosis and effective treatment. This study aims to access the plasma proteomic profile of patients with gout, CPPD disease, and healthy controls in order to identify potential biomarkers that can differentiate between these conditions.

Materials
Proximity extension assay technology (Olink) was used to measure 92 inflammation-related proteins in 71 patients with gout (GOUTROS), 86 patients with CPPD disease (COLCHICORT), and 96 elderly healthy controls (EHC). Potential biomarker was validated through in vitro experiments using human peripheral blood mononuclear cells (PBMCs).

Results
Comparison between gout and CPPD disease flare revealed 13 downregulated and 2 upregulated proteins, including, CASP-8 (FDR 3.30x10-18), FGF-5 (4.25x10-5), and TGF-α (1.0x10-4), CCL28 (0.001), AXIN1 (0.005) and 4E-BP1 (0.04). Notably, CASP-8 and 4E-BP1 were also differently expressed in EHC. To explore the potential of these proteins in distinguishing between gout, CPPD disease, and EHC, a multinomial logistic regression model adjusted for age and sex was performed. As result, CASP-8 and 4E-BP1 presented an area under the curve (AUC) above 0.97 and 0.63 for all 3 cohorts, respectively. Of importance, CASP-8 variation was not correlated with age and sex ($p \leq 0.001$). Moreover, the expression levels of CASP-8 were found to be elevated in all stages of gout including flare, intercritical, and after patients reached treat-to-target, in comparison with CPPD flare and EHC. To investigate the contribution of CASP-8 to MSU-induced inflammation, we exposed human PBMCs to Z-IETD-FMK, an irreversible CASP-8 inhibitor, followed by stimulation with MSU crystals + LPS. We observed that the production of proinflammatory cytokines, such as IL-6, TNF and IL-1β, significantly decreased in the presence of the inhibitor.

Conclusions
Our findings collectively indicate significant differences in systemic inflammation between patients with gout and CPPD disease. Of the 76 proteins tested, 19.7% exhibited differential expression during a gout or CPPD flare. Notably, CASP-8 has potential as biomarker for differentiating between gout and CPPD disease, besides playing an important role in the inflammatory response observed in patients with gout. However, whether CAPS-8 could also be used to differentiate between gout and hyperuricemia still needs further investigation.

Keywords
Inflammation, arthritis, diagnosis, therapy

DIFFERENCES IN BCG-INDUCED TRAINED IMMUNITY IN YOUNG AND OLD MICE

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Objectives
Trained immunity is defined by the ability of innate immune cells to develop memory traits and exhibit enhanced responsiveness to subsequent challenges through epigenetic and metabolic reprogramming1. In old age, the immune system's capacity to generate and sustain effective defense responses diminishes, resulting in an increased susceptibility to infections2. Whether induction of trained immunity is diminished in old age is not known. This study aimed to investigate the impact of aging on BCG's ability to trigger trained immunity.

Materials
Young (8-10 weeks old) and aged (8-14 months old) C57BL/6 mice were administered BCG (750 ug/mouse) intravenously, and 7 days later were infected i.v. with Candida albicans (2x105 CFU/mouse). Fungal burden was assessed in the liver and kidneys 24 hours post-infection by plating tissue homogenates on agar plates and enumerating colony-forming units (CFUs). Next, kidneys were harvested, fixed in formalin, and embedded in paraffin. Tissue sections were stained with periodic acid–Schiff (PAS) to visualize fungal structures and assess histopathological damage. Hyphal outgrowth of C. albicans was evaluated by microscopy. In addition, spleens were harvested from mice 7 days post-BCG vaccination. Splenocytes were isolated and stimulated ex vivo with lipopolysaccharide (LPS) or heat-killed Staphylococcus aureus for 48 hours. Cytokine levels (IL-1β, IL-6, TNF, and IFN-γ) in culture supernatants were quantified using enzyme-linked immunosorbent assay (ELISA).

Results
BCG vaccination significantly reduced C. albicans CFUs in the liver and kidneys of both young and older mice, but the effect was stronger in young mice. Histological analysis from kidneys of BCG-treated mice revealed extensive hyphal outgrowth of C. albicans in older mice compared with the young group. Additionally, while IL-1β, IL-6, and TNF production were similar between BCG-vaccinated older and young mice, IFN-γ induction was significantly higher in young mice vaccinated with BCG, highlighting an age-related difference in heterologous responses to microbial stimuli.

Conclusions
These findings suggest that BCG vaccination can induce trained immunity and increase the chances of survival against lethal infections in both young and older mice. However, the protective heterologous effects induced by BCG vaccination were significantly lower in older mice, highlighting the need for age-specific strategies to enhance vaccine efficacy in older populations.

Keywords
aging, Bacillus Calmette-Guérin (BCG), vaccination, infection, trained immunity

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REPROGRAMMING INNATE IMMUNE MEMORY USING TACROLIMUS-LOADED NANOBIOLOGICS PROMOTES ORGAN TRANSPLANT ACCEPTANCE

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Objectives
Tacrolimus is an anti-rejection drug primarily recognized for its immunosuppressive effects on T cells. We recently uncovered its tolerizing effects on innate immune memory. This memory involves long-lasting reprogramming of myeloid cells in hematopoietic organs and is thought to critically affect transplant survival [1-3]. Here, we investigated the effects of myeloid-directed tacrolimus-loaded nanobiologics (Tac-NBs) on innate immune memory and their potential to induce long term graft acceptance.

Materials
Human PBMCs stimulated with heat-killed C. albicans (HKCA) with or without Tac-NBs were evaluated in a trained immunity model assessing cytokine production and metabolism. Biodistribution and myeloid specific targeting was assessed in monkeys, mouse allogeneic heart transplant recipients and human PBMCs using 89Zr-labelled Tac-NBs and PET-CT imaging or fluorescently labeled Tac-NBs and flowcytometry. C57BL/6J mice transplanted with BALB/c hearts received PBS, empty NB, bare tacrolimus, Tac-NBs and/or CTLA4 Ig induction therapy. Interferon gamma (IFNy) producing cells were evaluated with an IFNy ELISPOT assay 6 days after transplantation, while graft survival was monitored for 100 days.

Results
Tac-NBs suppressed HKCA-induced IL-6/TNF production and metabolic activity upon restimulation in PBMCs. Tac-NBs predominantly accumulated in hematopoietic organs, lymph nodes, the liver and kidneys in monkeys and mice, with lower levels detected in the mice's grafts. Uptake of Tac-NB was observed in myeloid cells, while T cells excluded the drug both in vitro and in vivo. In the heart transplant mouse model, Tac-NBs induction treatment decreased IFNy-producing splenocytes and drastically improved graft survival compared to untreated controls (median 75 days vs 8 days, $p < 0.0001$). Long term graft acceptance (>100 days) was achieved in 62.5% of the mice when Tac-NB was combined with a single dose of CTLA4 Ig.

Conclusions
Tac-NBs can target the myeloid cells in hematopoietic organs in vivo and induce innate immune tolerance in vitro. In a heart transplant mouse model, Tac-NBs decrease reactive T cells and markedly extend allograft survival. Tac-NBs can achieve long-term graft acceptance when combined with a myeloid-T cell co-stimulation blocker (CTLA4-Ig). These findings demonstrate that short-term tacrolimus-induced reprogramming of innate immune cells can control the adaptive immune response and offers a promising approach to promote durable graft acceptance.

Keywords
Organ transplantation, innate immune memory, nanotechnology

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TARGETING IMMUNE CHECKPOINTS IN DIET-INDUCED INNATE IMMUNE MEMORY TO TREAT ATHEROSCLEROSIS

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Objectives
Atherosclerotic cardiovascular disease remains the leading cause of mortality worldwide and underlies acute adverse events such as stroke and myocardial infarction. Innate immune memory, a phenomenon in which innate immune cells mount enhanced, nonspecific responses following prior stimulation, has been implicated in exacerbating atherosclerosis. A Western-type diet is a potent inducer of innate immune memory and can thereby accelerate atherosclerotic progression. Here, we employ a validated murine model of diet-induced innate immune memory to investigate whether targeting innate immune checkpoints can modulate this process. We performed extensive immune profiling to characterize the bone marrow of mice subjected to a Western-type diet. Flow cytometric analysis of immune cell subsets and progenitors revealed a pro-inflammatory state driven by expansion of myeloid-biased multipotent progenitor populations compared to controls. Further characterization using single-cell RNA sequencing and single-cell ATAC sequencing identified differentially regulated immune pathways that may serve as therapeutic targets. The mammalian target of rapamycin (mTOR) and interleukin-1 (IL-1) pathways emerged as particularly relevant and feasible points of intervention to inhibit diet-induced innate immune memory. To this end, we have developed nanotherapeutics with diverse payloads to selectively target these pathways in an immunotherapeutic manner and thereby attenuate atherosclerosis in mice. Finally, we will investigate the impact of diet-induced innate immune memory in the context of myocardial infarction to uncover novel mechanisms amenable to therapeutic intervention.

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SEX-SPECIFIC DIFFERENCES IN INNATE IMMUNE PHENOTYPE IN AORTIC STENOSIS

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Objectives

Aortic stenosis (AS), prevalent in Western populations, involves fibrocalcific remodeling of the aortic valve, leading to obstructed ventricular outflow (1). Growing evidence implicates the innate immune system as a contributor to AS pathophysiology, promoting both fibrotic and calcific valve remodeling (2). AS presents differently between sexes: men predominantly exhibit a calcific phenotype, whereas women show a more fibrotic phenotype of the aortic valve. Women reach similar hemodynamic AS severity with less aortic valve calcification (3-6). The mechanisms driving these sex-specific differences remain unclear. This study aimed to elucidate sex-specific differences in innate immune phenotype and function that might contribute to AS development.

Materials

This multicenter case-control study included 119 patients with tricuspid AS (70% men) and 65 healthy controls (52% men). Blood composition, circulating inflammatory markers, and monocyte phenotypes were analyzed, with cytokine production evaluated after ex vivo PBMC stimulation. Additionally, aortic valves were obtained from 23 patients (61% men) undergoing elective surgical aortic valve replacement, for histological analyses.

Results

Male patients with AS exhibited a significantly higher area percentage of valve calcification (37.3 ± 12.0 vs. 25.3 ± 11.2, p = 0.025), whereas female patients showed higher proteoglycan percentages (9.3 ± 7.1 vs. 3.6 ± 2.4, p = 0.006) and a more pronounced pro-fibrotic phenotype. Male patients with AS also showed higher circulating monocyte percentages (10.4 ± 2.5 vs. 9.0 ± 2.2, p = 0.005), while female patients had higher lymphocyte percentages (31.0 ± 8.7 vs. 26.4 ± 8.2, p = 0.007). PBMC stimulation revealed significantly higher cytokine production of TNF-α, IL-1β, IL-6, and IL-1Ra in male patients with AS compared to females. Compared to female controls, female patients had lower platelet counts, higher median fluorescence intensity (MFI) for CD41 and C-C chemokine receptor 2 (CCR2), and lower MFI for HLA-DR. In males, only CCR2 MFI was significantly higher compared to their controls. Proinflammatory cytokine production was higher in male patients with AS than controls, while in female patients, mainly IL-1Ra was significantly higher.

Conclusions

This study highlighted sex-specific differences in AS, with men exhibiting a more calcific valve phenotype and women showing higher proteoglycan levels. Female patients with AS showed lower platelet counts and higher $CD41^{+}$ monocyte MFI compared to controls, while males displayed a more pronounced cytokine production. These findings suggest differential inflammatory mechanisms contributing to AS pathophysiology in men and women.

Keywords
Aortic Stenosis; Sex Differences; Innate Immune System

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HEPHESTOS - HEREDITARY PHEOCHROMOCYTOMA AND PARAGANGLIOMA ASSESSMENT TUMOUR IMMUNOLOGIES

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Objectives

Pheochromocytoma and paragangliomas (PPGLs) are rare neuroendocrine tumours arising from cells in the adrenal medulla or from extra-adrenal paraganglia. PPGL have the highest rate of heritability among all tumours, with around 30-35% of patients harbouring germline mutations in susceptibility genes while another 30-40% of patients harbour a somatic driver mutation. Surgical resection of the primary tumour is the first-choice treatment of PPGL. While approximately 70% of all patients can be assigned into three different molecular clusters, cluster-specific personalized treatment has not entered routine clinical practice for inoperable, recurrent, or metastatic disease. Historically, PPGL have been considered as immunologically 'cold' tumours. However, more recent research has emphasized the immunogenic nature of PPGL, highlighting the tumour microenvironment (TME) and circulatory factors as key components. This study aims to examine the genotypes' effect on the immune system, focusing on the immune cell composition in the circulation and in the TME.

Materials

For this study 80 patients with PPGL, 80 carriers of germline mutations predisposing for PPGL, and 40 sex and age matched healthy volunteers will be included. Blood will be analysed from patients before and after surgical resection of the primary tumour, and 1 and 2 years after surgery. Primary outcomes will be the inflammatory molecules and proteins produced by stimulated and unstimulated immune cells from circulation, immune cell composition in histological PPGL samples and in circulation, and their genetic determinants. Secondary outcomes will comprise of transcriptional and epigenetic signature of circulating immune cells, circulating immunomodulating metabolites, trained immunity, and clinical outcomes such as tumour metastasis, tumour recurrence, and survival.

Results

This is an abstract on future research, and no results have been generated yet.

Conclusions

This is an abstract on future research, and no conclusions have been generated yet.

Keywords

Endocrinology, adrenal gland, pheochromocytoma, paraganglioma

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QDATA – AN OPEN-SOURCE SOFTWARE FOR AUTOMATIC QRT-PCR DATA ANALYSIS

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Objectives
Currently, quantitative real-time PCR (qRT-PCR) is the golden standard for targeted gene expression experiments and the Livak formula is the most popular implementation for calculating fold change values starting from cycle threshold (Ct) measurements. In the Drosophila Laboratory of Faculty of Biology, University of Bucharest, we developed qDATA, an open-source bioinformatics software designed for functional genomics research applications by performing automated analysis of qRT-PCR data.

Materials
The qDATA software is freely available to download from GitHub (https://github.com/DL-UB/qDATA) under CC0-1.0 Universal license.

Results
qDATA provides a streamlined workflow with a modern and user-friendly graphical interface, enabling users to perform descriptive statistics, assess data normality and conduct statistical testing on $2^{-\Delta Ct}$ (or ΔCt) and fold change values calculated with the Livak's method. qDATA. The algorithm implements a standardized strategy of performing all possible ΔCt values within a biological replicate.
The software requires a standard input table, which can be easily created by the user based on the output generated by the qRT-PCR machine. qDATA can tackle qRT-PCR experiments with any number of genes of interest, any number of biological replicates (BRs) and any number of technical replicates (TRs), including unequal numbers of BRs and TRs between different samples.
qDATA automatically performs all calculations fully offline in a matter of seconds. All tables and graphic representations created during the analysis process can be exported as publication-ready. Various calculations are also available to export and may be used as input for statistics programs. The software's code is written in the R programming language and employs the shiny package, enabling multiple instances of qDATA to run in parallel in order to analyze multiple datasets. It is compatible with all major operating systems including Windows, macOS or Linux, and does not require programming experience.

Conclusions
Currently, qDATA was successfully used to analyze qRT-PCR data published in two ISI rated journals.

Keywords
qDATA, software, qRT-PCR, gene expression, R

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DSCAFF: A BIOINFORMATICS TOOL FOR REFERENCE-GUIDED SCAFFOLDING

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Objectives
In the context of rapid advancements in sequencing technologies and the resulting flow of genomic data, the need for efficient genome assembly and scaffolding tools has become increasingly important. To address these challenges, we developed the digital Scaffolding (dScaff) application. dScaff enables the construction of customized scaffolds by leveraging a reference genome to guide the annotation, ordering, and filtering of contigs forming a de novo assembly.

Materials
dScaff encapsulates a suite of scripts written in R and Bash, available at https://github.com/DL-UB/dScaff. The methodology relies on the BLAST heuristic and offers two operational strategies: gene queries, which utilizes annotated gene sequences and their genomic coordinates, and ranked queries, which employs regularly distributed sub-sequences extracted from the reference genome using a dedicated script (https://github.com/DL-UB/SubSequencesExtractor).
In order to run dScaff, the user requires the contig-level assembly of interest, a FASTA file containing the query sequences, and a table with the corresponding query coordinates according to the reference genome. The main output is a FASTA file containing an ordered and annotated selection of contigs forming a so-called minimal scaffold.

Results
The effectiveness of dScaff in reducing redundancy while preserving assembly quality was demonstrated across multiple model organisms. For example, a Drosophila melanogaster assembly initially contained 99.7% complete BUSCO genes, of which 1.6% were duplicated. When dScaff applies the ranked and gene queries strategies, the proportion of duplicated genes decreased by 0.9% and, respectively, by 1.2%. In a D. suzukii assembly, the proportion of duplicated genes was reduced by 8.2%, with only a minor 3.6% decrease of the complete genes parameter. For a Bombyx mori assembly, the number of duplicated genes dropped by 52%, coupled with a 5.5% reduction of complete genes.

Conclusions
Overall, dScaff provides a fast, user-friendly and effective solution for reference-guided scaffolding while simultaneously reducing redundancy in draft genome assemblies. We intend to improve the computing performances of dScaff in order to make it more adaptable when dealing with big mammalian genomes. As a fact, a more accurate assembling process generates a more reliable platform for any consecutive functional genomic studies.

Keywords
dScaff, R, bash, sequencing, scaffolding

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SYSTEMS BIOLOGY APPROACHES TO UNVEIL THE UNDERLYING SUSCEPTIBILITY TO INVASIVE PULMONARY ASPERGILLOSIS

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Objectives

Patients undergoing immunosuppressive treatments are especially at risk of acquiring invasive pulmonary aspergillosis (IPA); however, not all patients develop this fungal infection (1). Given the variable risk of infection among patients with similar clinical conditions, susceptibility to IPA is thought to be largely influenced by genetic predisposition. Previous research has identified single-nucleotide polymorphisms (SNPs) in immunity genes that render patients highly susceptible to IPA (2).

Our project aims to map the genetic architecture of antifungal immunity and develop innovative strategies for the personalised management of IPA by linking genome-wide interindividual variation to molecular and cellular signatures of the host-fungus interaction and defining risk score algorithms with potential clinical applicability.

Materials

We have elected macrophages as our model system due to their well-established role in the clearance of infection. From a cohort of ~ 500 healthy donors of European ancestry recruited at Hospital de Braga, blood will be collected, and after isolation and differentiation of monocytes into macrophages, these will be stimulated with Aspergillus fumigatus, and the fungicidal activity of macrophages will be assessed using a live-cell imaging analysis.

We will employ the Illumina Genome Screening Array v3.0 for genotyping. To identify QTLs, we will associate different functional parameters at each time point post-infection with proximal and distal SNPs using previously published methodologies.

For metabolomic analysis, blood serum samples will be collected from donors, and metabolite levels will be measured. To identify genetic determinants of metabolites linked to IPA, mQTL (metabolite quantitative trait loci) mapping will be done using the R package Matrix-eQTL.

The downstream annotation of these functional QTLs will also involve colocalization with expression of QTLs, allowing us to prioritise candidate genes and potential genetic mechanisms explaining the variability in fungicidal capacity of macrophages.

Results

We anticipate the following outcomes:

1. Generation of an atlas of genetic variation regulating the molecular basis of antifungal immunity
2. A genomic map of antifungal immunity in susceptibility to IPA
3. Mechanistic insights into the genes and pathways contributing to patient-specific causal mechanisms

Conclusions

This study will provide insights into how genetic differences impact metabolite levels, shape immune phenotypes and impact disease susceptibility in the context of IPA and ultimately predict the risk of infection and druggable targets.

Keywords
Antifungal immunity, Genome-wide association analysis, mQTL mapping, and Metabolomics

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EXPLORING ACTIONABLE VULNERABILITIES IN LUSC THROUGH TARGETED COMPOUND SCREENING

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Objectives
Lung squamous carcinoma (LUSC), a subtype of non-small cell lung cancer (NSCLC), is often associated with poor prognosis because of the lack of actionable targets and the high resistance to conventional therapies. Immuno-oncology compounds targeting key pathways such as angiogenesis, cell cycle regulation and DNA damage and repair (DDR), offer potential therapeutic strategies. This study employs transcriptomic profiling in LUSC cell lines, aiming to identify cytotoxic immuno-oncology compounds and evaluate their cellular and molecular effects.

Materials
Gene expression profiling of two untreated LUSC cell lines (H1703 and SK-MES1) was conducted using microarray technology, following RNA extraction. To refine the selection of candidate compounds, an integrated bioinformatics approach was applied, comparing microarray data from the two LUSC cell lines with publicly available TCGA data from LUSC patient samples. This analysis identified 233 commonly altered genes across all three datasets, distributed across multiple dysregulated cellular pathways. Based on these findings, 27 compounds from a commercially available Immuno-Oncology Compound Library were screened on the two cell lines. Compounds with cytotoxic effects on both cell lines were selected, identifying five active compounds. Cytotoxicity was assessed using RealTime-GloTM MT Cell Viability Assay to determine IC50 values. Functional tests including cell cycle analysis and apoptosis assay were performed on Nexcelom's Celigo platform and RT-qPCR was done on downstream pathway effectors of the selected compounds for functional pathway modulation.

Results
Functional tests and gene expression analysis were performed on two of the five compounds and chosen for the presentation of this poster. In the cell cycle assay, ENMD-2076 induced G_2/M arrest in both SKMES1 and H1703, reflecting its known Aurora A–mediated disruption of mitotic progression—consistent with reports in glioblastoma and colorectal cancer models. Conversely, bosutinib caused an accumulation in sub-G_0/G_1 phase in SKMES1, indicative of apoptosis and growth arrest, mirroring outcomes seen in CML models. In scratch assay, bosutinib significantly inhibited SKMES1 migration—aligning with documented migration blockade via ACK1 inhibition in NSCLC—whereas ENMD-2076 achieved stronger suppression in SKMES1, likely through MMP9 downregulation and EMT inhibition. Apoptosis assay revealed elevated caspase activity with ENMD-2076 across both cell lines and potent induction of programmed cell death, paralleling findings from glioblastoma and breast

cancer studies. The observed cellular responses align with the known mechanisms of action of the selected compounds, reinforcing their potential therapeutic relevance in LUSC.

Conclusions

Our findings show that both bosutinib and ENMD-2076 exert multi-dimensional anticancer effects beyond gene expression changes. Our results confirm that bosutinib promotes migration inhibition, while ENMD-2076 enforces mitotic arrest, invasion suppression, and cell death—reflecting the mechanistic patterns observed in analogous cancer models and suggesting complementary therapeutic actions in LUSC.

Keywords

screening ; functional tests ; functional genomics ; gene analysis

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FUNCTIONAL GENOMICS OF IMMUNE REGULATION IN SOLID CANCERS: A PAN-CANCER BIOINFORMATIC ANALYSIS OF TUMOUR ANTIGEN EXPRESSION

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Objectives
Tumor antigens play a crucial role in immune recognition and are actively investigated as targets for cancer immunotherapy. However, their expression and immunogenic potential vary across tumor types and are influenced by the surrounding immune microenvironment [1,2]. This study aims to characterize the expression of immune-related tumor antigens across diverse solid cancers using a pan-cancer bioinformatic approach.

Materials
We analyzed RNA-seq gene expression datasets from The Cancer Genome Atlas, which cover approximately 20 solid tumor types, including lung, thyroid, and renal cancers. A curated panel of tumor-associated and tumor-specific antigens was evaluated using R-based differential expression analysis. Immune landscape features were explored through enrichment and deconvolution methods.

Results
Preliminary analysis reveals cancer type–specific expression of antigens such as MAGE-A1, NY-ESO-1, CEACAM5, ERBB2, and WT1. Tumors with high antigen expression often exhibit increased levels of immune checkpoints and infiltration of suppressive immune cells, such as Tregs and MDSCs, suggesting compensatory immune evasion mechanisms.

Conclusions
Our in silico pan-cancer profiling highlights the complexity of tumor antigen biology and immune suppression in solid tumors. These findings may inform future immunotherapeutic strategies by identifying candidate antigens and guiding the development of combinatorial targeting approaches.

Keywords
Functional genomics; Immune regulation; Pan-cancer analysis, Bioinformatics

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DUAL TRANSCRIPTOME–PROTEOME ANALYSIS REVEALS DISTINCT IMMUNE ENDOTYPES IN SEPSIS

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Objectives
1. Validate IFNγ-CXCL9/CXCL10 biomarkers Confirm the diagnostic and prognostic value of IFNγ-CXCL9/CXCL10 axis activation in an independent sepsis cohort to establish its reliability for endotype classification.
2. Characterize Clostridium-specific immune signatures Define the unique transcriptomic features that distinguish Clostridium sepsis from other gastrointestinal infections to understand pathogen-specific host responses.

Materials
Within the Functional Genomics in Severe Infections (FUSE) project, we enrolled 125 adults with Sepsis-2–defined sepsis and 299 healthy volunteers. Ninety-two plasma inflammatory proteins were quantified; unsupervised hierarchical clustering of differentially expressed analytes yielded two endotypes, "high-" and "low-inflammatory." Peripheral-blood mononuclear cells from the same participants underwent bulk RNA-seq. Differential expression was assessed with DESeq2, and enriched pathways were identified by over-representation analysis. Transcriptomic profiles were then compared across infection sites (pneumonia, urinary tract, and gastrointestinal), with a focused sub-analysis of culture-confirmed Clostridium cases.

Results
Sepsis prompted widespread gene-expression remodeling: pathways mediating phagocytosis and antimicrobial-peptide synthesis were markedly up-regulated, whereas NK-cell cytotoxic programs and adaptive-immune pathways linked to T-cell signaling were defective. Proteomics-defined endotypes a high-inflammatory endotype carrying a heavier cytokine burden and more severe organ dysfunction. Within this high-inflammatory endotype, the IFN-γ–responsive chemokines CXCL9 and CXCL10 were among the most up-regulated transcripts and displayed high cytokine concentrations in plasma. Transcriptomic patterns were remarkably consistent across pneumonia, urinary-tract and gastrointestinal-origin sepsis, indicating that disease severity, not infection site, dominates the host response. Finally, culture-confirmed Clostridium cases displayed an additional transcriptomic signature that set them apart from other gastrointestinal infections.

Conclusions
In sepsis, immune dysregulation is driven chiefly by disease severity rather than by the infection's origin or pathogen. The pronounced activation of the IFNγ–CXCL9/CXCL10 axis in the high-inflammatory endotype therefore stands out as a compelling target for precision, host-directed treatment.

Keywords
Sepsis endotypes, IFNγ-CXCL9/CXCL10 axis, Multi-omics integration, Transcriptomics, Inflammatory proteomics Host-directed therapy, Precision immunotherapy, Immune dysregulation, Clostridium sepsis, Biomarker stratification

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Dual Transcriptome–Proteome Analysis Reveals Distinct Immune Endotypes in Sepsis

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Background. Sepsis remains a global health crisis, driving about 49 million cases and 11 million deaths each year. Persistent failures of trials testing immunotherapies highlight the need to stratify patients into discrete immune endotypes and tailor treatment accordingly. Herein, we mapped the systemic immune landscape of sepsis, profiled site-specific transcriptomic signatures, paying special attention to *Clostridium*-driven disease, and integrated blood transcriptomics with targeted inflammatory proteomics to identify novel multi-omic endotypes.

Methods
Within the Functional Genomics in Severe Infections (FUSE) project, we enrolled 125 adults with Sepsis-2–defined sepsis and 299 healthy volunteers. Ninety-two plasma inflammatory proteins were quantified; unsupervised hierarchical clustering of differentially expressed analytes yielded two endotypes, "high-" and "low-inflammatory." Peripheral-blood mononuclear cells from the same participants underwent bulk RNA-seq. Differential expression was assessed with DESeq2, and enriched pathways were identified by over-representation analysis. Transcriptomic profiles were then compared across infection sites (pneumonia, urinary tract, and gastrointestinal), with a focused sub-analysis of culture-confirmed *Clostridium* cases.

Results
Sepsis prompted widespread gene-expression remodeling: pathways mediating phagocytosis and antimicrobial-peptide synthesis were markedly up-regulated, whereas NK-cell cytotoxic programs and adaptive-immune pathways linked to T-cell signaling were defective. Proteomics-defined endotypes a high-inflammatory endotype carrying a heavier cytokine burden and more severe organ dysfunction. Within this high-inflammatory endotype, the IFN-γ–responsive chemokines CXCL9 and CXCL10 were among the most up-regulated transcripts and displayed high cytokine concentrations in plasma. Transcriptomic patterns were remarkably consistent across pneumonia, urinary-tract and gastrointestinal-origin sepsis, indicating that disease severity, not infection site, dominates the host response. Finally, culture-confirmed *Clostridium* cases displayed an additional transcriptomic signature that set them apart from other gastrointestinal infections.

Conclusions
In sepsis, immune dysregulation is driven chiefly by disease severity rather than by the infection's origin or pathogen. The pronounced activation of the IFNγ–CXCL9/CXCL10 axis in the high inflammatory endotype therefore stands out as a compelling target for precision, host-directed treatment.

ELEVATED INFLAMMATORY MARKERS ARE ASSOCIATED TO CLINICAL PHENOTYPES IN ANTIPHOSPHOLIPID SYNDROME

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Objectives
Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by the persistent presence of antiphospholipid antibodies (aPL) in patients with thrombotic events and/or obstetric complications. aPL induce a pro-inflammatory and prothrombotic state in endothelial cells, neutrophils and monocyte, making APS a paradigmatic thromboinflammatory disease. Recurrent thrombotic events despite adequate anticoagulation are common in APS. This means it could be of great benefit to understand the inflammatory side of this disease. We used targeted proteomics in APS patients and healthy controls to investigate which inflammatory proteins play a role in APS.

Materials
We included 142 APS patients and 65 healthy controls, matched for age and sex. 86 APS patients had a thrombotic phenotype, 22 had an obstetric phenotype, and 31 had both. A targeted proteomics panel consisting of 92 inflammatory proteins was performed in EDTA plasma (Olink Inflammation). Protein expression patterns were compared between the different subgroups using a principal component analysis (PCA), and differences were tested using a Wilcoxon signed-rank test with correction for multiple testing.

Results
Levels of 28 circulating inflammatory proteins were higher in APS patients than healthy controls (FDR < 0.05), including IL6, TNF, and type I interferon related proteins like CCL19, CXCL9, CXCL10. CXCL5, AXIN1, and ST1A1 were lower in APS patients. (Figure 1B). A thrombotic phenotype was positively associated with 27 inflammatory proteins (including TNF, IL6, CCL2, CCL19, CXCL9, CXCL10 (FDR <0.05) (Figure 1C). Patients with an obstetric phenotype had higher levels of 11 inflammatory cytokines in comparison to healthy controls, including IL10, IL-10RA, TNF receptor SF9, and TNFB. (FDR <0.05) (Figure 1D). Higher circulating levels of TNF were found in patients positive for lupus anticoagulans (LAC, a marker for disease severity) (FDR <0.05) (Figure 1E).

Conclusions
Both thrombotic and obstetric APS demonstrate an overall increase in inflammatory protein concentrations compared to healthy controls, with the most pronounced increase in thrombotic patients. LAC positivity, which is related to disease severity in thrombotic patients, showed a distinct association with higher circulating TNF levels compared to LAC negative patients. These data support the hypothesis that APS patients exhibit a proinflammatory phenotype.

Keywords
antiphospholipid syndrome, inflammation, proteomics

ITF3756 EXHIBITS INHIBITORY EFFECTS IN ANAPLASTIC THYROID CARCINOMA

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Objectives
Anaplastic thyroid carcinoma (ATC) is a rare but highly aggressive thyroid malignancy characterized by rapid proliferation, early metastasis, high mutational burden, and extreme therapy resistance. Despite accounting for less than 3% of thyroid cancers, ATC presents with a median survival of under six months and remains underrepresented in clinical trials. This highlights a pressing need to identify new therapeutic strategies.

Materials
Transcriptomic profiling was performed to compare gene expression patterns between anaplastic thyroid carcinoma (ATC) cell lines (C643 and CAL-62) and normal human thyroid epithelial cells (HPTE). Differential gene expression analysis was conducted to identify significantly upregulated and downregulated genes. Based on the results, a targeted screening was performed using selected compounds from the Small Molecule Immuno-Oncology Compound Library (Selleckchem, Library No. L4800). Among the screened molecules, ITF3756—a selective histone deacetylase 6 (HDAC6) inhibitor—was selected for further evaluation. Functional validation assays included apoptosis detection, mitochondrial membrane potential assessment, cell cycle analysis, and gene expression profiling via RT-qPCR.

Results
Differential expression analysis identified 2164 genes as upregulated and 2425 as downregulated in ATC cell lines compared to normal HPTE cells. Several therapeutic compounds were screened for selective cytotoxicity in ATC cell lines. Functional assays confirmed that ITF3756 induced apoptosis, caused mitochondrial depolarization, and triggered cell cycle arrest in ATC cells. RT-qPCR analysis revealed that ITF3756 modulated the expression of NF-κB pathway-related genes, indicating partial suppression of inflammatory signaling pathways in tumor cells.

Conclusions
In conclusion, our study underscores the relevance of transcriptomic profiling in uncovering novel treatment opportunities for anaplastic thyroid carcinoma and supports ITF3756 as a promising therapeutic candidate for further development in the treatment of ATC.

Keywords
anaplastic thyroid carcinoma, transcriptomic profiling, small molecule screening, ITF3756

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STRESS-INDUCED INNATE IMMUNE CELL REPROGRAMMING IN CARDIOVASCULAR DISEASE: DESIGN OF AN OBSERVATIONAL COHORT STUDY

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Objectives
Cardiovascular diseases (CVD) including stroke, myocardial infarction, and peripheral arterial disease, are the main cause of mortality worldwide. The majority of CVD is caused by atherosclerosis, which is a chronic low-grade inflammatory disorder of the arterial wall. Innate immune cells, including monocytes and macrophages, importantly contribute to the pathophysiology of atherosclerosis. Epidemiological studies have well-established that psychosocial stress is an independent risk factor for atherosclerotic CVD [1]. Preclinical studies have suggested that stress leads to a prolonged hyperresponsiveness of innate immune cells by (epigenetic) reprogramming of their bone marrow progenitor cells, but this has never been tested in humans [2,3]. In this study, we aim to investigate the effects of psychosocial stress on the innate immune system in patients with heightened risk for CVD.

Materials
85 patients with a heightened cardiovascular risk will be included in this observational cross-sectional study. Patients will fill out questionnaires, and donate blood and hair. Stress will be quantified by well-validated questionnaires and by measurement of hair cortisol. The perceived stress scale-10 (PSS10) will be used to assess stress exposure in the last month, and the Stress and Adversity Inventory (STRAIN) for life long stress exposure. The primary outcome is the ex vivo cytokine production by isolated PBMCs. Additional study parameters focus on extensive phenotyping of circulating immune cells with the use of flow cytometry, and (single cell) RNA and ATAC sequencing.

Results
We hypothesize that stress exacerbates atherosclerotic disease through epigenetic modifications that underlie long-term increased functional responsiveness of innate immune cells, known as trained immunity.

Keywords
Atherosclerosis, stress, innate immune system

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DELIVERING CYTOKINE MRNA TO SECONDARY LYMPHOID ORGANS FOR ROBUST CANCER IMMUNOTHERAPY

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Objectives
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Materials
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Results
Efficient delivery of nucleic acid therapeutics to immune cells is essential for robust immunomodulation for cancer therapy. In our recent study1, we developed apolipoprotein A1 nanoparticles (aNPs) that encapsulate small interfering RNA (siRNA) targeting hematopoietic stem and myeloid progenitor cells.
Leveraging the same design principles, we engineered aNPs that stably incorporate messenger RNA (mRNA) together with our proprietary dendrimer-based ionizable lipids. Radiotracer biodistribution and PET-CT imaging showed a preferential uptake of aNP-HMJ1 in the secondary lymphoid organs, including spleen and lymph nodes. A screening with mCherry-mRNA showed that compared to clinically applied benchmark lipid nanoparticles (LNP), aNP-HMJ1 produced 3-fold less protein in off-target hepatocytes while producing more protein in splenic monocytes, aligning with our goal of regulating immunity in secondary lymphoid organs.
Replacing mCherry- with human IL2-mRNA (hIL2-mRNA) yielded sustained serum exposure and high hIL2 concentrations in target organs, the spleen and lymph nodes. hIL2 was selected for its ability to expand and activate CD8 T cells, thereby potentiating their cytotoxic anti-tumor effect.
In treatment studies, we showed significantly reduced tumor growth and increased overall survival in the B16F10 melanoma mouse model, and achieved complete tumor remission in the MC38 colorectal tumor mouse model. To demonstrate the translational capacity of our aNP platform, we radiolabeled aNPs to track their biodistribution in non-human primates using PET-MRI, confirming high splenic uptake when dosed with either mCherry- or hIL2-mRNA. We confirmed functional translation by detecting mCherry in myeloid cells and measuring elevated hIL2 levels in serum.

Conclusions
In summary, our aNP platform enables cytokine mRNA delivery to immune cells and secondary lymphoid organs in mice and non-human primates, and abrogates tumor growth in mice, highlighting its potential as a next-generation immunotherapy.

Keywords
mRNA therapeutics, cytokine therapy, cancer therapy, PET imaging

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MULTI-OMICS ANALYSIS ON HOST AND HIV RESERVOIR HETEROGENEITY REVEALS THREE DISTINCT CLUSTERS: DIFFERENCES IN TOTAL AND INTACT HIV RESERVOIRS ARE ASSOCIATED WITH DISTINCT IMMUNE MECHANISMS

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Objectives
People with HIV (PWH) on suppressive antiretroviral therapy exhibit heterogeneity in HIV reservoir quantity and quality [1]. Multi-omics integration can reveal molecular and immunological patterns underlying this variability [2]. This study aimed to identify clusters of PWH with distinct viral reservoir and molecular profiles, characterize immunological features, explore clinical associations, and identify predictive markers in the 2000HIV cohort.

Materials
Multi-omics data from 1,230 PWH in the 2000HIV cohort (NCT03994835) [3] were analyzed, split into discovery (n=1,027) and validation (n=203) sub-cohorts. Data included bulk transcriptomics (58,347 genes, PBMCs), DNA methylation (793,775 CpG-sites, PBMCs), plasma proteomics (2,367 proteins, Olink), immune phenotyping (355 markers, whole blood), and cytokine production (90 markers, PBMCs ex-vivo stimulation). Total and intact HIV DNA were quantified in CD4+ T cells (DNA copies/million CD4 T cells). After outlier removal (n=39), the MoCluster algorithm enabled integrative clustering and marker identification [4], followed by predictive modeling (XGBoost/SHAP) [5, 6] to assess feature importance. Single-layer analyses revealed broader cluster differences among baseline and stimulated immune cells, adjusting for confounders (e.g., age, sex). Comorbidity associations were tested statistically.

Results
Three consistent clusters were identified in both sub-cohorts, with the reservoir as a key contributor: Low-Total Low-Intact (LTLI, n=428), High-Total Low-Intact (HTLI, n=483), and High-Total High-Intact (HTHI, n=280). Key clustering markers included elevated TCF7 expression (CD4+ T-cells), IFI44L hypermethylation, and lower SLAMF7 protein levels in LTLI ($P<0.05$), among others. TCF7 and IL-1β (upon LPS and CMV 24h-stimulation) were ranked among top predictive features of total reservoir differences between clusters, and IFN-γ for intactness, consistent with immune regulation roles in HIV persistence [7]. Differential analysis revealed that total reservoir differences (LTLI vs. HTLI/HTHI) associated with baseline differences in immune markers and comorbidities, including lower CD8+ T-cell activation/exhaustion, enrichment of naïve/regulatory CD4+ subsets, and lower incidence of cardiovascular disease in LTLI ($P<0.05$) [8]. Intactness differences (HTHI vs. HTLI/LTLI) were linked to functional immune responsiveness, with higher overall IFN-γ production upon 7-day stimulation in individuals with lower intact proviral burden (HTLI/LTLI) [9].

Conclusions

Multi-omics clustering identified three distinct groups, including an HTLI cluster, suggesting differential regulation of total and intact reservoir. Higher reservoir total mainly associated with baseline immune changes, such as exhaustion/activation, and higher cardiovascular comorbidities. In contrast, lower intactness linked to enhanced IFN-γ–mediated responses. These findings reveal human host-virus interplay, offering insights for personalized HIV cure strategies targeting total and intact reservoir.

Keywords

HIV reservoir; Multi-omics; Integrative clustering; Immune mechanisms; Total HIV DNA; Intact HIV DNA; Comorbidities; HIV cure; Personalized Medicine

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EPIGENETIC CLOCKS REVEAL ACCELERATED AGING IN PATIENTS WITH GOUT AND INDIVIDUALS WITH HYPERURICEMIA

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Objectives
Epigenetic clocks are predictive models trained on DNA methylation at CpG sites to estimate biological age, offering insights that may differ from an individual's chronological age. These tools are central in aging research, enabling the assessment of age-related health and disease risk. Hyperuricemia is a metabolic disorder that plays a key role in the onset of gout. This study aims to explore whether hyperuricemia and gout are associated with accelerated biological aging by analyzing DNA methylation patterns and immune cell proportions in whole blood of individuals with normouricemia, asymptomatic hyperuricemia, and gout.

Materials
DNA methylation profiles were obtained using the Infinium EPIC v2 array across three groups: 150 normouricemic controls, 128 individuals with asymptomatic hyperuricemia, and 148 patients diagnosed with gout. Beta values were extracted with the getBeta function from the preprocessFunnorm package in R. Epigenetic age was estimated using three established DNA methylation clocks: Horvath, Hannum and PhenoAge. DNA methylation age (DNAmAge) was estimated using the methylclock package in R. Epigenetic age acceleration (EAA) was calculated as the residuals from regressing DNAmAge on chronological age. Blood cell counts were inferred from DNA methylation data with FlowSorted.Blood.EPIC.

Results
Horvath DNAmAge was strongly correlated with chronological age across all groups. However, no significant differences in cell-intrinsic epigenetic age acceleration were observed among any of the groups (one-way ANOVA, followed by adjustment for multiple pairwise comparison). Using the Hannum clock to estimate systemic biological aging, gout patients showed significantly higher epigenetic age acceleration compared to controls (mean difference: 1.86 years, $p = 0.016$). Using the PhenoAge clock, both gout and hyperuricemia were associated with significantly increased biological age acceleration compared to controls (mean differences: 3.63 years, $p = 0.012$ for gout; 4.7 years, $p = 0.002$ for hyperuricemia). Significant shifts in immune cell composition were also observed. Gout and hyperuricemia were associated with reduced CD4+ and CD8+ T cells, and increased neutrophil proportions, compared to controls. Monocyte levels were elevated in gout versus normouricemia. Across all groups, chronological age and DNAmAge estimates (Horvath, Hannum, PhenoAge) showed significant inverse correlations with CD4+ and CD8+ T cell proportions, and positive correlations with neutrophil cell proportions. Linear modeling incorporating interaction terms (lm(EAA ~ Cells * Group)) indicated that neutrophil and monocyte proportions were significantly associated with epigenetic age acceleration (EAA) as measured by the Horvath clock in individuals with gout ($p = 0.010$ and $p = 0.018$, respectively), and monocytes were also associated

with EAA in hyperuricemia ($p = 0.004$). Using the Hannum clock, monocyte proportions remained significantly associated with EAA in gout ($p = 0.0106$), with a trend observed for neutrophils ($p = 0.0666$). Notably, both neutrophil ($p = 0.00015$) and monocyte ($p = 0.027$) proportions were significantly linked to PhenoAge (Levine) EAA in gout. In contrast, no significant interactions were found in the hyperuricemia group for Hannum or PhenoAge clocks.

Conclusions

These findings suggest that biological aging may be accelerated in disease states such as gout and hyperuricemia. Shifts in neutrophil and monocyte composition may contribute to or reflect intrinsic epigenetic aging processes. Given the well-established influence of sex and smoking on aging processes, future analyses should incorporate sex and smoking as covariates in the regression models and explore sex-stratified patterns of epigenetic aging.

Keywords

Epigenetic clocks, Accelerated Aging, DNA methylation, Gout, Hyperuricemia

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MSU CRYSTALS SYNERGIZE WITH PALMITATE TO INCREASE THE PRODUCTION OF IL-1β WITHOUT ANY SIGNIFICANT TRANSCRIPTIONAL CHANGES IN HUMAN PBMCS

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Objectives
The deposition of monosodium urate crystals (MSU) crystals in joints is the main trigger of gouty inflammation. Multiple studies have investigated how immune cells interact with MSU crystals, often with seemingly contradictory results[1,2,3]. The main objective of this study is to characterize the Peripheral Blood Mononuclear Cell (PBMCs) response to MSU crystals and other relevant stimuli.

Materials
Fresh PBMCs were isolated and stimulated with Palmitate, MSU crystals and combination of both for 24 hours. Cytokine production was then measured in 2 independent cohorts by ELISA in 181/132 controls and 123/148 gout patients. For the first cohort, an experiment with LPS and LPS in combination with MSU was additionally performed.
A second pair of experiments with identical conditions was performed and followed by a RNA-sequencing analysis. The first included 4 gout patients, 3 of which were reanalyzed after a month, forming a validation group. The second analysis included 4 healthy donors, therefore obtaining 3 transcriptomic datasets. We consider to be differentially expressed, the genes with padj <= 0.05 and log2 Fold Change >= 0.58 across all datasets.

Results
IL-1β production was significantly increased when PBMCs were stimulated with Palmitate and even stronger in Palmitate+MSU combination. No significant differences in IL-1β production between the LPS and LPS+MSU groups were observed. We find no Differentially Expressed Genes in any of the following analyses: MSU vs Control, Palmitate+MSU vs Palmitate, nor LPS+MSU vs LPS.

Conclusions
MSU crystals have a synergistic effect with palmitate that leads to higher IL-1β production, which cannot be found in combination with LPS. At the same time, the crystals did not influence the PBMC transcriptome alone nor in combination with other stimuli. We conclude that MSU crystals are not involved in TLR4 signaling and the increase in IL-1β production is most likely caused by NLRP3 inflammasome activation.

Keywords
Monosodium Urate Crystals, Interleukin-1, transcriptomics, palmitate, LPS

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MODULATING DIET-INDUCED TRAINED IMMUNITY IN ATHEROSCLEROSIS USING NANOBIOLOGICS

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Objectives
1. Validate a diet-induced trained immunity atherosclerosis model in ApoE -/- mice
2. Investigate the ability of nanobiologics containing prodrugs to modulate atherosclerosis-like trained immunity in vitro
3. Evaluate the potential of nanobiologics as therapeutic interventions for atherosclerosis in vivo

Materials
To investigate the role of diet switches on trained immunity in atherosclerosis, ApoE^{-}/$^{-}$ mice were fed a trained immunity-inducing diet consisting of alternating periods of high-fat and conventional chow, designed to mimic fluctuating metabolic stress and promote innate immune activation. Atherosclerotic lesion development was evaluated through histological staining and immunohistochemistry to assess plaque volume and inflammation to validate the in vivo model.
In parallel, the potential of nanobiologics to modulate trained immunity in atheroclerosis was explored using an in vitro training assay. Nanobiologic formulations containing prodrugs targeting key trained immunity pathways, such as Tacrolimus and other modulators, were tested on human peripheral blood mononuclear cells (PBMCs). Cells were primed with oxidized LDL (Ox-LDL) or heat-killed Candida albicans in the presence or absence of nanobiologics, followed by restimulation with LPS. Cytokine production was measured via ELISA to identify reduction of training.

Results
Preliminary findings suggest that nanobiologics may modulate and reduce the induction of trained immunity in vitro, although further validation is required to confirm these effects. Histological analysis of ApoE^{-}/$^{-}$ mice fed a high-fat diet confirmed the expected increase in atherosclerotic plaque formation, validating the diet-induced model. However, no significant differences in overall plaque burden were observed between the standard high-fat diet group and the trained immunity-inducing diet group at this stage. Additional immunohistochemical staining is ongoing to assess potential differences in plaque composition, immune cell infiltration, and features associated with plaque stability.

Conclusions
These initial results indicate that while the model successfully induces atherosclerosis, the specific contribution of trained immunity-inducing diets to plaque progression remains to be clarified. Nanobiologics show promise in modulating trained immunity, but further in vitro and in vivo studies are needed to fully understand their therapeutic potential. Future work will focus on deeper characterization of plaque composition, immune cell phenotypes, and the in vivo effects of nanobiologics, as well as extending the approach to immune responses in tissue-engineered vascular grafts.

Keywords
Trained immunity - atherosclerosis - Nanobiologics - Myeloid cells

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PERINATAL IMPAIRED CYTOKINE RESPONSE IN AT RISK PREGNANT WOMEN. THE MERSIN STUDY (MYELOID DERIVED SUPPRESSOR CELLS – ROLE IN SEVERE INFECTIONS)

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Objectives
Pregnancy requires fine immunological tuning in order to protect mother and fetus, while ensuring antimicrobial protection. The physiological immune response may be altered in high-risk perinatal conditions—premature rupture of the membranes, chorioamnionitis and pre-eclampsia, with the overall result of marked inflammatory and cytotoxic effects. The MERSIN study aimed to characterize immune response changes in normal and high-risk pregnancies, and to identify immune markers predicting perinatal sepsis in the mother and/or neonate.

Materials
The MERSIN study included 18 pregnant women admitted to the Department of Obstetrics and Gynecology of the County Clinical Emergency Hospital Craiova, classified by either normal or high-risk pregnancies. Clinical data were collected using standardized interview forms. Samples were collected at 3 timepoints of the perinatal timeline. PBMCs were isolated from whole blood, and stimulated with clinically relevant sepsis pathogens or with purified PAMPs (pathogen-associated molecular patterns) derived from these pathogens, for 24 hours and for 7 days. The immune response was quantified using ELISA (Enzyme-Linked Immunosorbent Assay), by measuring relevant pro- and anti-inflammatory cytokines.

Results
Preliminary analysis indicates peak levels of IL-6 in the period before birth, in the presence of bacterial stimuli, for high-risk pregnancies, when compared to those with a normal course. Interestingly, a reverse image is registered for IL-1beta levels. The trend would argue for a more pro-inflammatory status in high-risk pregnancies before and immediately after birth.

Conclusions
Less physiological immunosuppression for pregnancies at risk was described around the moment of birth. Characterizing these divergent cytokine landscapes may reveal mechanisms driving perinatal complications and identify interventions directed to restore immune equilibrium.

Keywords
myeloid-derived suppressor cells, perinatal sepsis

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Perinatal impaired cytokine response in at risk pregnant women. The MERSIN study (Myeloid dErived suppressor cells – Role in Severe INfections)

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Background.
Pregnancy requires fine immunological tuning in order to protect mother and fetus, while ensuring antimicrobial protection. The physiological immune response may be altered in high-risk perinatal conditions—premature rupture of the membranes, chorioamnionitis and pre-eclampsia, with the overall result of marked inflammatory and cytotoxic effects. The MERSIN study aimed to characterize immune response changes in normal and high-risk pregnancies, and to identify immune markers predicting perinatal sepsis in the mother and/or neonate.

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Preliminary analysis indicates peak levels of IL-6 in the period before birth, in the presence of bacterial stimuli, for high-risk pregnancies, when compared to those with a normal course. Interestingly, a reverse image is registered for IL-1beta levels. The trend would argue for a more pro-inflammatory status in high-risk pregnancies before and immediately after birth.

Conclusions.
Less physiological immunosuppression for pregnancies at risk was described around the moment of birth. Characterizing these divergent cytokine landscapes may reveal mechanisms driving perinatal complications and identify interventions directed to restore immune equilibrium.

Synergy Among Epithelial, Monocyte, and CD8 T Cell Triad Enhances Antiviral Immunity

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Abstract

Trained immunity induced by malaria infection enhances resistance to subsequent bacterial infections; however, its role in viral immunity remains largely unexplored. This study elucidates the unexpected cross-protective effects of prior malaria infection against respiratory viral infections, particularly influenza A virus (IAV) and respiratory syncytial virus (RSV). Utilizing spatiotranscriptomics and single-cell RNA sequencing (scRNA-seq), we reveal that monocytes, traditionally viewed as crucial for antibacterial responses, serve as critical immune sentinels in this context. They identify virus-infected epithelial cells and act as a pivotal anchor for the immune response. In response, these monocytes secrete chemokines, notably CCL5 and CCL4, recruiting CD8 T effector memory cells (Tem) to the site of infection via CCR5 signaling. Importantly, while monocytes are necessary for an antiviral response, our findings demonstrate that the elevated levels of CD8 Tem in prior malaria-infected individuals are a prerequisite for enhanced antiviral activity. The recruited CD8 Tem cells are recruited by monocyte. Notably, the protective effect was abolished in CCR2 knockout (KO) mice and CD8 T cell-depleted mice, underscoring the necessity of these pathways. Furthermore, adoptive transfer of CD8 Tem from malaria-recovered mice significantly enhances antiviral efficacy in recipient cells. Together, this synergistic interaction among infected epithelial cells, monocytes, and CD8 Tem cells significantly restricts viral replication, as evidenced by reduced viral load, and mitigates pulmonary damage. Our findings illuminate the intricate interplay among these cell types in orchestrating antiviral immunity, highlighting the essential role of the triad interaction in achieving effective immune protection and offering insights that could inform innovative therapeutic strategies to bolster host defenses against respiratory viruses.

Comprehensive Immunophenotyping of Age-Related Variability in Circulating Immune Cell Subsets Following an Inactivated Influenza Vaccine and an Adjuvanted Recombinant Herpes Zoster Vaccine

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Abstract

With age, the immune response is remodelled in a process called immunosenescence, marked by an imbalance in the naïve-to-memory cell ratio, impaired T and B cell function, dysregulated innate responses, and chronic low-grade inflammation. These factors contribute to infection susceptibility, age-related disease onset, and poor vaccination efficacy in older adults, as seen with influenza vaccines. Interestingly, the AS01B-adjuvanted herpes zoster vaccine (RZV, Shingrix) maintains high efficacy in older adults. To identify potential factors underlying this disparity in vaccine efficacy between younger and older individuals, we conducted a randomized, partially placebo-controlled, open-label clinical study. Young adults (18-35 years of age, n=84) were randomized 3:3:1:1 to RZV, an unadjuvanted quadrivalent influenza vaccine (IIV4, Fluarix Tetra), or their respective placebos. Older adults (≥60, n=63) were randomized 1:1 to receive RZV or IIV4. To investigate the contribution of alterations in circulating immune cell composition and function, this study employed three flow cytometry panels. These panels phenotypically and functionally identified major circulating innate and adaptative immune cells, assessing their functional status in terms of activation, exhaustion, and maturation.

Baseline evaluation of immune cell populations showed lower proportions of circulating Vδ2+ γδ T cells, pDCs, and CD8+ T cells in older individuals, compared to young adults, and higher proportions of CD16+ mDCs, NK-T cells, CD4+ T cells, and NK cells. RZV vaccination resulted in similar trends in circulating immune cell population over time in both young and older individuals, with a decrease in neutrophils, eosinophils, CD14+ and CD16+ monocytes, and an increase in CD4+ and CD8+ T cells, and γδ T cells. IIV4 vaccination resulted in only minor changes in immune cell populations in young individuals, whereas in older individuals IIV4 vaccination increased the frequency of B cells, NK cells, and γδ T cells, and decreased the relative abundance of CD14+ monocytes and CD1c+ mDCs. In addition, we observed higher expression of PD-L1 on monocytes, B cells and dendritic cells 60 days after IIV4 vaccination in older individuals, but not in the young group. Our results indicate that advanced age may be associated with variations in immune responses to vaccination, and that a deeper understanding of the underlying mechanisms is necessary to optimize vaccination strategies and overcome immunosenescence in older individuals.

Distinct circulating circulating myeloid cell signatures as potential novel treatment targets in anaplastic thyroid cancer

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Objectives: Anaplastic thyroid cancer (ATC) is an extremely aggressive malignancy with limited treatment options and very poor survival, so there is an urgent call for new treatment strategies for this disease. In the ATC tumor-microenvironment, pro-tumoral immune cells like tumor-associated macrophages are very abundant, however, circulating immune cells of ATC patients have not yet been fully immunophenotyped. In this study, we aimed to characterize circulating immune cells of ATC patients, which could result in new targets for future treatment strategies.

Methods: Patients with ATC, poorly differentiated thyroid cancer, differentiated thyroid cancer and healthy controls were recruited between September 2022 and March 2025 and donated blood. Immune cells were isolated and we assessed cytokine production capacity by ELISA, surface marker expression by flow cytometry, epigenome by ATAC-sequencing and transcriptome by single-cell RNA-sequencing. In addition, circulating inflammatory proteins were assessed in plasma using Olink and ELISAs.

Results: In whole blood, neutrophil and monocyte counts were higher in the ATC groups compared to the other subgroups. After 7 days of *ex vivo* stimulation, lymphocytes produced less interferon-γ than the other subgroups. In contrast, 24 hours of stimulation resulted in higher concentrations of monocyte-derived cytokines IL-6 (interleukin-6), IL-8 and IL-1Ra in the ATC subgroup. Circulating monocytes of ATC patients were relatively more often of the pro-tumoral non-classical subtype and less often of the classical subtype. The epigenetic and transcriptomic profiles of monocytes from ATC patients were distinct from those of the other groups with upregulated pro-inflammatory and proangiogenic pathways. In plasma, circulating IL-6, IL-1Ra and granulocyte colony stimulating factor were higher in the ATC group than in the other subgroups, which could explain the high levels of neutrophils and monocytes in ATC patients. The role of the IL-6/JAK/STAT3-pathway in ATC tumor cell proliferation was validated *in vitro;* several inhibitors of this pathway significantly decreased ATC tumor cell survival in multiple cell lines.

Conclusion: Circulating myeloid cells of ATC patients show distinct pro-inflammatory pro-tumoral phenotypes. Targeting and reprogramming these cells towards an anti-tumoral phenotype could be exploited as potential novel treatment strategy for these patients.

Long- and short read transcriptomics reveals novel genes and transcripts in the human immune response

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Background. Immune responses are shaped by the nature of infections and by inter-individual variability, contributing to di@erential susceptibility to infections and to various diseases with an inflammatory component. Dynamic transcript and protein expression in a range of cells responsible for the innate immune response is important to shape the first line of defense against a wide variety of pathogens.

Methods. We perturbed immune-cells with *in vitro* pathogen exposure. We stimulated PBMCs from 5 healthy donors for 4h or 24h with LPS, *S. aureus*, Poly(I:C) or *C. albicans* with RPMI medium as control. We performed short read sequencing (Lexogen QuantSeq) in all samples and long read sequencing using PacBio IsoSeq in a subset of samples. We separately performed long read single cell sequencing using MAS-IsoSeq in a separate sample of Poly(I:C)-stimulated and RPMI-cultured PBMCs from a single individual.

Results. Short-read sequencing reveals common and distinct genes and pathways expressed during immune responses to di@erent pathogens, the results of which can be interactively explored at emilvorsteveld.shinyapps.io/app_de/. Beside well-established genes, we highlight uncharacterized genes *KIAA0040* and *FAM49A,* di@erentially expressed upon pathogen exposure and co-expressed with modules of established immune genes. Long-read sequencing revealed that
47.7% of all transcripts are novel, i.e. new use of exon combinations, novel exons, as well as e.g. intron retention transcriptomes. We found widespread isoform switching induced upon pathogen stimulation. We highlight novel transcripts of *NFKB1 and CASP1* that may indicate novel immunological mechanisms. We find a substantial number of di@erentially expressed transcripts upon Poly(I:C) stimulation using long read single cell sequencing, highlighting the expression of a novel *CCL2-CCL8* transcript in monocytes.

Conclusion. Transcriptome profiling of pathogen-stimulated immune cells using paired short- and long-read approaches highlights candidate immune genes and identifies novel transcripts, revealing a more complex transcriptome landscape following pathogen exposure than previously appreciated.

Serum inflammatory and metabolic profilesofpost-treatment Lyme disease syndrome (PTLDS)

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Background. Lyme borreliosis(LB)is caused by the tick borne spirochete *B. burgdorferisl*, and is the most common vector-borne disease in North America, Europe and Asia. LB generally has a good prognosis after treatment, however a large number of patients with LB still report symptoms >6 months after infection.This is called post-treatment Lyme disease syndrome (PTLDS)and is characterizedby symptoms includingfatigue, musculoskeletalpain, cognitiveimpairmentand an overall reduced qualityof life.So far, the biological mechanismunderlying PTLDS remainsunknown. With this project we aimed to investigate the difference in circulating inflammatory proteins and metabolites in people withPTLDS and recovered controls.

Methods. We included 50 people from the LymeProspect cohort(NL50227.094.14). All 50 participants had LB at baseline, of which 30 participantsrecovered and 20participantsdeveloped PTLDS. Serum samples were collected at baseline and 6 weeks post-treatmentand were used to measure the Olink proteomics inflammation panel (consisting of 92 inflammatory proteins) and for untargeted metabolomics. Furthermore, extensive clinical assessmentsand symptomsurveys were collectedof all participants.

Results. Sixweeks after infection, 5 proteins (IL-17C, CCL20, FGF-19, EN-RAGE and TRAIL) were higherin the recovered group compared to baseline($p<0.05$), which was not seen in the PTLDS group. At baseline, NRTN was higher in the recoveredgroup($p<0.05$). After 6 weeks, 4 proteins (FGF-19, FGF-23, FGF-5 and NT-3) were higher in the recovered group compared to the PTLDSgroup ($p<0.05$). One metabolite, Lucuminamide, was higher at baseline in the recovered group (FDR<0.05).

Conclusion. Overall our results suggesta differenceininflammatory profile in people with PTLDS, mainly visible6 weeks after infection. Lower NRTN in the PTLDS groupduring infectionpoints toward a role of neuronsurvival andneurondifferentiationin the development of PTLDS. We identified several potential biomarkers of PTLDSwhich should be confirmed byfuture research.

Male-Specific Plasma Immune Signatures in HIV Associate with Viral Reservoir Size

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Introduction Biological sex influences HIV-specific and clinical outcomes in people with HIV (PWH) receiving antiretroviral therapy (ART). Notably, females have a lower reservoir size, and an increased HIV-induced cardiovascular disease (CVD) risk. To better understand these diYerences, we compared the plasma proteome in virally suppressed males and females with HIV and assessed associations with viral reservoir size and CVD.

Methods. Targeted plasma proteomics of 2367 proteins was performed in two independent cohorts of ART-treated PWH from the 2000HIV study (Discovery: 1,232 males, 213 females, Validation: 262 males, 46 females). Associations between the plasma proteome and sex were assessed using linear regression. HIV-specificity of the sex-biased diYerentially expressed proteins (DEPs) was assessed by comparison to DEPs from a population-based study (UK Biobank). Total and intact viral reservoir were measured in circulating CD4 T cells using the Rainbow assay. Associations of sex-specific DEPs with reservoir size and CVD were assessed in the 2000HIV cohort, and overlaid with publicly available CVD associations from the UK Biobank in males and females separately.

Results. We identified 484 sex-biased DEPs, mostly overlapping with the UK Biobank. Twenty-four sex-DEPs were HIV specific, of which twenty-three were lower in females, mostly related to NK and T cell function. Three of these (CXCL9, TNFRSF9 and KLRD1) were positively associated with the total or intact reservoir size. While no HIV- and sex-specific DEPs were associated with CVD in our cohort, eighteen associated with CVD in the UK Biobank in either sex. This included proteins involved in platelet and endothelial activation, such as higher VWF and lower ANGPT1 in females, associated with higher CVD risk, while lower levels of immunological proteins (CXCL9, IL10 and TNFRSF9) appeared protective.

Conclusions. As in the general population, we found extensive sex diYerences in the plasma proteome of virally suppressed PWH. Notably, twenty-four HIV- and sex-specific DEPs suggest increased immune activation in males, three of them linked to larger reservoir size. The relationship between HIV- and sex-specific DEPs and CVD was more complex, as some may promote disease while others seem protective. This suggests that sex diYerences may influence outcomes of interventions in virally suppressed PWH.

Residual HIV viremia more than doubles cardiovascular disease incidence independent of classical cardiovascular risk factors in a prospective Dutch cohort of people with HIV

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Introduction. Antiretroviral therapy (ART) inhibits HIV replication resulting in plasma HIV-RNA levels below the lower level of detection of commonly used PCR tests. However, HIV transcription from latently infected cells may still occur and result in unquantifiable low plasma HIV-RNA levels, called residual viremia (RV). Furthermore, despite ART, people with HIV have an excess risk in cardiovascular disease (CVD), estimated around 2-fold, a phenomenon not fully explained by traditional cardiovascular risk factors. We hypothesize that residual viremia increases the risk for CVD.

Methods. We utilized data from the 2000HIV study, a prospective cohort study enrolling 1895 virally suppressed people with HIV between 2019-2021 in the Netherlands. Participants were stratified based on the presence of RV or undetectable viral load (TND) at baseline. Extensive clinical and multi-omics characterization was performed at inclusion. Incident CVD was registered after 2-year follow-up. Multivariate logistic regression models, adjusting for classical CVD risk factors, were used to estimate adjusted odds ratios (aOR) for incident CVD associated with RV.

Results. One-third of study participants had RV, RV independently and strongly increased the risk of developing a first cardiovascular event (3.1% vs. 1.2%, aOR 2.61, p=0.012). Conventional CVD risk prediction tools, such as SCORE2, underestimated CVD risk in individuals with RV by over two-fold. The elevated CVD risk was not explained by markers of immune activation, gut barrier dysfunction, systemic inflammation, or lipometabolic alterations, suggesting a direct impact of HIV or its proteins on vascular plaque stability.

Conclusion. Residual viremia is a strong, independent predictor of cardiovascular events in people with HIV using ART. These findings support incorporating RV into CVD risk prediction models and exploring novel prevention strategies targeting HIV-related mechanisms.

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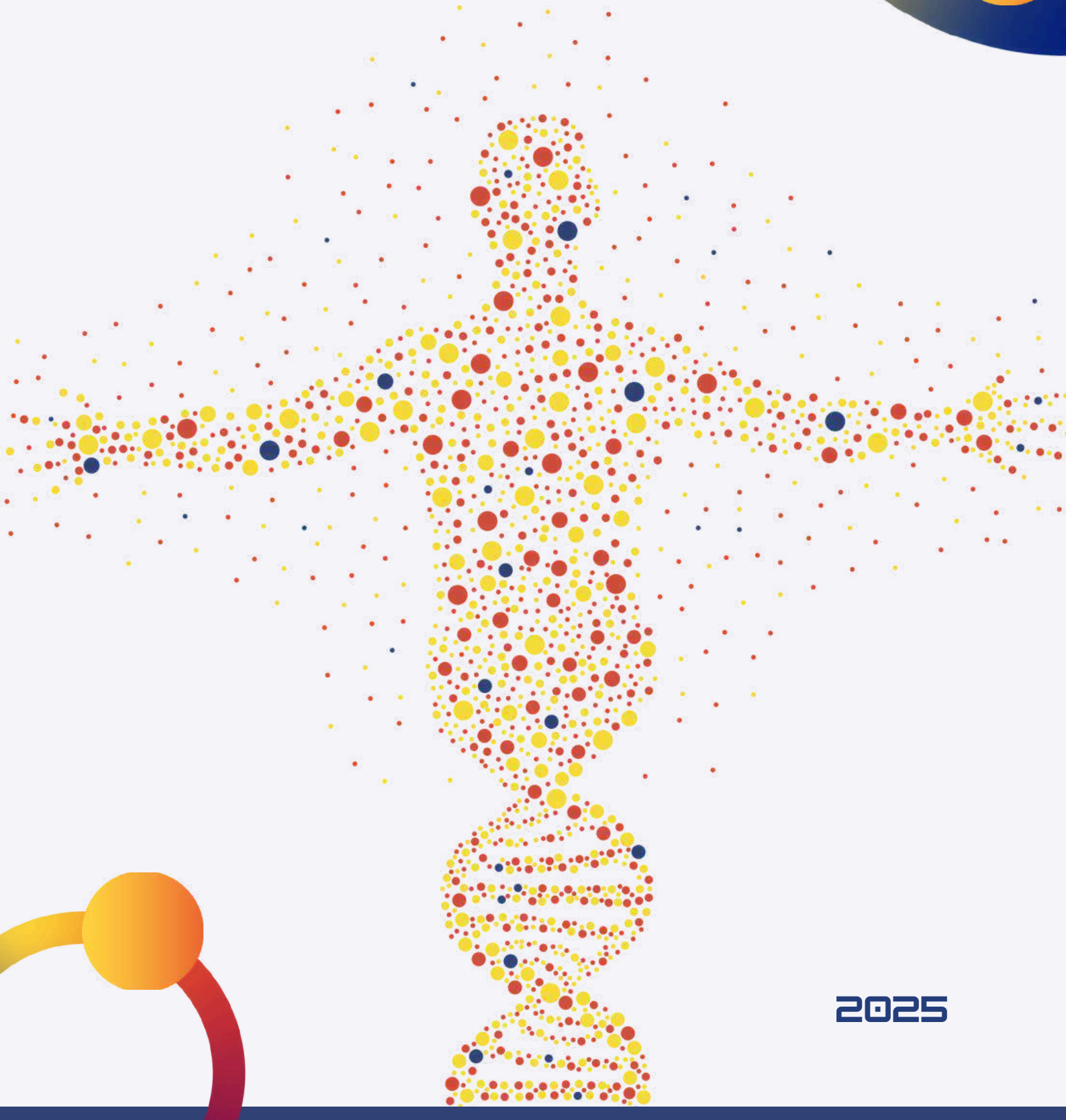

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