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# ABSTRACTS

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# SYK SIGNALING LINKS VIRAL IMMUNE COMPLEXES TO NLRP3 INFLAMMASOME ACTIVATION IN MACROPHAGES

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**Objectives**

The inflammasome is a vital component of innate immunity, with NLRP3 as the best-described inflammasome. NLRP3 inflammasome activation triggers the release of proinflammatory cytokines, such as IL-1β, and induces pyroptosis. Spleen tyrosine kinase (SYK), a non-receptor kinase involved in immunoreceptor signalling, has been implicated in regulating NLRP3 inflammasome activation. Our previous research demonstrated that viral antigens and their immune complexes (IC) trigger NLRP3 inflammasome activation in macrophages. However, limited data are available on IC-induced signalling related to the NLRP3 inflammasome and its relationship to macrophage functions, such as phagocytosis and antigen presentation. Therefore, this study aimed to determine SYK role in the IC-induced NLRP3 inflammasome activation pathway and macrophage effector functions.

**Materials and Methods**

Primary murine microglia were treated with spherical virus-like particles (VLPs) of human WU polyomavirus and their IC formed with different murine IgG subtypes. NLRP3 inflammasome activation was assessed by measuring IL-1β and TNF-α cytokine release and ASC speck formation. Specific inhibitor R406 was used to inhibit SYK activity and define its role. Protein expression and activation were analysed by Western blot, and phagocytosis with antigen presentation were measured by flow cytometry.

**Results**

It was found that VLPs and their ICs activate SYK, while R406 blocks SYK activation, NLRP3 expression, cytokine secretion, and ASC speck formation in microglia, indicating inhibition of NLRP3 inflammasome activation. IC mediated a higher cellular response than VLPs alone. The results also revealed that SYK signalling is required for antigen presentation induced by ICs.

**Conclusions**

In conclusion, our study demonstrates that IC can enhance the inflammatory response in microglia via SYK dependent pathway.

# Neuroimmunology

# ALTERED GUT BARRIER AND IMMUNE ACTIVATION BIOMARKERS IN PATIENTS WITH RELAPSING–REMITTING MULTIPLE SCLEROSIS

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**Objectives**

Multiple sclerosis (MS) is a chronic immune-mediated disorder in which peripheral immune dysregulation contributes to central nervous system inflammation. Growing evidence implicates the gut–immune axis in MS pathophysiology, linking alterations in gut microbiome composition with impaired intestinal barrier integrity and increased microbial translocation. Circulating markers such as lipopolysaccharide-binding protein (LBP), soluble CD14 (sCD14), zonulin, occludin, and immune activation markers including CD27, chitinase-3-like protein 1 (CHI3L1), and interferon-γ (IFN-γ) may reflect these processes.

The objective of this study was to characterise gut microbiome composition and markers of intestinal permeability, microbial translocation, and systemic immune activation in MS patients.

**Materials and Methods**

In this pilot study, 65 patients with relapsing–remitting multiple sclerosis (RRMS; 16 males, 49 females; median age 35.5 years, range 19–67 years) were enrolled. An age and sex matched group of healthy individuals served as controls. Plasma concentrations of LBP, occludin, zonulin, sCD14, CD27, CHI3L1, and IFN γ were quantified using commercially available ELISA kits. Gut microbiome profiling was performed on RRMS samples using high throughput paired end sequencing (PE150) on the DNBSEQ G400 platform (MGI Tech Co., China), achieving an average sequencing depth of approximately 15 million paired end reads per sample.

**Results**

Plasma levels of zonulin (median 258.2 vs 112.9 ng/mL; $p = 0.005$), sCD14 (1716 vs 1494 ng/mL; $p = 0.0418$), CD27 (1675 vs 1212 pg/mL; $p < 0.001$), and IFN γ (172.7 vs 98.92 pg/mL; $p < 0.0001$) were significantly higher in patients with RRMS compared with controls. In contrast, occludin levels were significantly higher in controls than in the RRMS group (6.22 vs 3.85 ng/mL; $p = 0.0074$). No significant difference was observed for CHI3L1 levels between RRMS and control groups (585.65 vs 49.02 ng/mL; $p = 0.1090$).

A total of 55 samples were subjected to metagenomic sequencing. The mean raw read count per sample was 20,232,063 ± 4,399,901. Following quality filtering and removal of human reads, an average of 19,322,369 ± 4,621,942 reads per sample were retained for taxonomic classification against the UHGG database. On average, 16,143,009 ± 3,822,302 reads (83.5% of raw reads) were classified as microbial. The most abundant genera were Prevotella (15.67%), Bacteroides (8.58%), Phocaeicola (8.45%), and Faecalibacterium (5.34%). Samples were rarefied to 2,000,000 reads per sample, yielding a mean Shannon diversity index of 4.50 ± 0.56.

Correlation analyses between gut microbiome composition, plasma biomarkers, and clinical characteristics are currently ongoing.

**Conclusions**

Patients with RRMS exhibited elevated plasma markers associated with intestinal barrier dysregulation, microbial translocation, and immune activation, alongside reduced levels of the tight junction protein occludin, compared with controls. These findings support the involvement of gut barrier alterations and systemic inflammatory responses in RRMS. Ongoing correlation analyses between gut microbiome composition, plasma biomarkers, and clinical characteristics will further elucidate microbiota–immune interactions in RRMS and their potential relevance to disease mechanisms.

# Immune Regulation and Tolerance

# REVERSAL OF IMMUNE EVASION IN NSCLC: THE ROLE OF DIOSPYROS PEREGRINA IN PROMOTING PRO-INFLAMMATORY TH LINEAGE SPECIFICATION

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**Objectives**

To study and explore the role of DFP-matured dendritic cells in TH cell differentiation in NSCLC.

To find out the association of DFP-matured dendritic cells with the signatory markers for different lineages of TH cells in NSCLC.

**Materials and Methods**

Diospyros peregrina fruit extract, Cancer cell lines

Dendritic cells (DCs) were pulsed with LCA in the presence or absence of DFP. analysed by RT-qPCR. Pulsed DCs were irradiated and co-cultured with autologous and allogeneic lymphocytes. Cytokine levels were estimated via ELISA from extracellular supernatants, and an LDH release assay was used to test Cytotoxic T lymphocytes (CTLs) mediated cytotoxicity.

**Results**

Cytotoxic T lymphocyte mediated anti-tumour response was greatly directed by the DFP treated LCA pulsed DCs.

DFP matured moDCs increased extracellular secretion of TNF-α, IFN-γ and IL-12, and decreased that of IL-23, IL-12 and CCL2

Increased levels of anti-tumorigenic cytokines like TNF-α, IFN-γ and IL-12 released as a result of DFP treatment suggest augmented TH1 lineage directed T helper cell differentiation

DFP results in upregulation of intracellular TBX21, projecting towards TH1 mediated immune response and STAT1 activation

**Conclusions**

DFP serves as a potent immunomodulator that evokes tumor-protective immunity against NSCLC by orchestrating a specific DC-T cell crosstalk that favors a robust Th antitumor response

# DECTIN-1 RECOGNITION OF B-GLUCANS: A MOLECULAR DOCKING STUDY

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**Objectives**

Analyze the interaction of branched and linear β-glucans with Dectin-1 homodimer using molecular docking.

**Materials and Methods**

Molecular docking experiments were done with linear and branched β-1,3-glucans. All ligands were prepared in CHARMM-GUI glycan reader and modelling module. Linear molecules consisted of 5, 7, 9, 11 and 15 glucose monomers. Length of β-1,6-glucan side-chain were: one, two, three, four monomers, and β-1,4-N-acetylglucoasmine – one and two monomers, attached to 5 monomer β-1,3-glucan backbone. 3D ligand models were optimized using the MFF force field in AVOGADRO (version 1.2.0). The structure of murine Dectin-1 homodimer (PDB ID: 2CL8) was prepared using PyMOL (version 2.5.4). Protein interaction domains were identified using PyMOL. Grid box with corresponding coordinates and 40 × 40 × 30 Å size (X, Y, Z), were prepared in AutoDockTools. Molecular docking was performed in GNINA (version 1.1) using a flexible ligand, a rigid protein, standard parameters and exhaustiveness value of 16. The LigPlot+ (version 2.2) was used to evaluate protein-ligand hydrogen bonds and hydrophobic interactions and the interaction data presented as heatmap.

**Results**

Based on the size of 1520 Å and structure of the binding pocket formed by Dectin-1 homodimer, estimated using PyMOL (v2.5.4) and validated with ProteinsPlus, assumption was made that binding pocket formed by Dectin-1 dimerization could accommodate both linear and branched β-glucans.

Docking results showed that the interaction between β-glucans and the Dectin-1 dimer is energetically favorable with Vina affinity of branched and linear β-glucans ranging from −11 kcal/mol to −13 kcal/mol.

Branching had minimal effect on the amino acid residues involved in the interaction. However, three or four side-chain units formed alternative binding modes. Docking with tetrameric β-1,6-glucan or dimeric β-1,4-linked chitin side chains revealed mostly energetically unfavorable binding.

LigPlot+ analysis showed that the interaction between protein and ligand involves 5 to 6 glucose residues of linear β-glucan. The docking conformations of the linear ligands were mostly similar. Docking of the branched ligand showed that the one and two glucose units side-chains could fit within the protein binding pocket alongside the β-glucan backbone. Regardless of conformation, length or branching, the most frequent interactions were observed with amino acids Ser148, Glu194, and Glu243 from both chains of the Dectin-1 dimer. Residues such as Arg145, Gln149, Gly151, Ala152 and Asp195, also contributed to ligand binding. Side-chains interacted with the same amino acids as the backbone and strengthened the interaction by forming additional hydrogen bonds. β-glucans with three- or four units of the β-1,6-side-chain adopted alternative conformation with side-chain entering the binding pocket and β-1,3-backbone remaining outside.

**Conclusions**

Molecular docking analysis showed that both linear and branched β-1,3-glucans are capable of interacting with Dectin-1 dimer. The interaction was mostly mediated by residues Ser148, Glu194 and Glu243 residues with additional involvement of Arg145, Gln149, Gly151, Ala152 and Asp195. During Dectin-1 oligomerization, homodimer formation could be intermediate state during an immune response against yeast cell wall components, which require interaction with linear β-glucans or branched β-glucans with short side-chains.

# Translational immunology

# ALPHAVIRUS DERIVED TRANS-AMPLIFYING RNA IS A VERSATILE PLATFORM FOR DEVELOPMENT OF VACCINES OTHER IMMUNE THERAPIES

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## Objectives

Alphaviral genomes are bicistronic mRNAs encoding RNA-dependent RNA-polymerase (replicase) in the first ORF and structural proteins in the second. By replacing the structural gene with any gene of interest and producing RNA via in vitro transcription, self-amplifying RNA (saRNA) is generated, a potent vector recently approved as SARS-CoV2 vaccine when encoding the spike protein. However, the coupling of replicase expression, RNA replication, and antigen production restricts engineering of the saRNA vector. To address this, we developed trans-amplifying RNA (taRNA), a bipartite mRNA platform consisting of a non-replicating mRNA encoding replicase and a second antigen-coding RNA that is replicated in trans by replicase once inside the cell.

I summarize our work on taRNA and present how the system's flexibility could accelerate vaccine production and lower costs.

## Materials and Methods

We utilized reporter gene expression analysis in tissue culture for vector optimization and qPCR to assess intracellular RNA replication. We monitored vector toxicity via cell viability assays and assessed activation of innate immunity by type-I interferon secretion. To evaluate immune responses to Influenza A virus (IAV) and Chikungunya virus (CHIKV), taRNA encoding corresponding antigens was administered to mice.

## Results

By separating replicase expression and antigen amplification into two molecules, we could manipulate the system in several ways. We simplified the trans-replicon RNA (TR-RNA) by removing the subgenomic promoter and redesigning the 5' untranslated region, generating a streamlined TR (STR) that can be directly translated and amplified by replicase. By combining taRNA and immune evasion genes from different alphaviral species we increased expression. We used directed evolution on STR to select for more efficient replication and adapted taRNA to tolerate N1-methylpseudouridine. We developed a replicase with enhanced activity to further boost taRNA expression. We showed that taRNA outperforms mRNA for reprogramming of fibroblasts into iPS cells and for expression of CAR and TCR in T cells.

In our CHIK-taRNA design, we significantly reduced the required dose. Neutralizing antibodies were induced with just 12 ng of taRNA, whereas in studies with mRNA encoding Chikungunya virus antigen 100-1000fold higher doses were required.

## Conclusions

We believe that taRNA holds great potential as a next-generation RNA vector for vaccines and other immune therapies by combining the benefits of viral vectors and mRNA technology.

# LONG-TERM IMMUNOLOGICAL, INFLAMMATORY, AND HEPATIC CHANGES FOLLOWING COVID-19: A 2.5-YEAR LONGITUDINAL FOLLOW-UP STUDY

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**Objectives**

To assess recovery trajectories from the acute phase to 2.5-year follow-up in patients with different initial COVID-19 severity, and to identify persisting immunological, inflammatory, and liver function biomarker alterations at follow-up.

**Materials and Methods**

A longitudinal observational study enrolled 74 patients hospitalized with COVID-19 of varying severity in 2020. Peripheral blood samples collected during the acute phase were compared with samples obtained at 2.5-year follow-up. Assessed parameters included immune cell counts (leucocytes, neutrophils, monocytes) and lymphocyte subsets ($CD4^+$, $CD8^+$, measured by flow cytometry), inflammatory markers (CRP, IL-6), liver enzymes (ALT, AST, GGT), and fibrosis markers (HA, M30-CK18). Additionally, liver stiffness and fat content were assessed by ultrasound-based techniques (SWE, ATI) at follow-up. Longitudinal changes were analyzed using nonparametric tests and recovery dynamics by disease severity (non-severe vs severe/critical) were assessed using mixed models. To evaluate long-term differences, patients were stratified at follow-up by disease severity and liver injury at admission; between-group comparisons were adjusted for age, sex, and BMI. Correlations between immune-inflammatory and liver markers were assessed.

**Results**

Most immunological, inflammatory, and liver function markers normalized over 2.5 years. Lymphocyte and $CD4^+$ T-cell counts increased approximately 2 times ($p < 0.001$), and CRP and IL-6 markedly decreased (from 29.4 to 2.1 mg/L and 11.65 to 2.00 pg/mL, respectively). Fibrosis markers HA and M30-CK18 also decreased approximately 2 times ($p \leq 0.001$). Significant group × time interactions were observed for lymphocytes, $CD4^+$ T cells, CRP, and AST (all $p \leq 0.016$), indicating differential recovery trajectories by severity. At 2.5-year follow-up, severe/critical patients had persistently higher total leukocyte counts (1.23 ×103/μL, $p = 0.020$), monocyte counts (0.14 ×103/μL, $p = 0.018$), $CD8^+$ T cells (70% higher, $p = 0.002$), and IL-6 (28% higher, $p = 0.046$) after adjustment. Patients with acute liver injury had higher $CD4^+$ T-cell counts (adjusted difference 227 cells/μL, $p = 0.016$) and higher ALT (23% higher, $p = 0.048$) at follow-up. Among immune cells, only $CD8^+$ T cells demonstrated moderate positive association with ATI. Among inflammation markers, IL-6 showed a moderate positive correlation with SWE and a weaker association with ATI, while CRP correlated positively with GGT and ALT.

**Conclusions**

Despite broad normalization of biomarkers, subclinical immunological and hepatic alterations persist 2.5 years after COVID-19, particularly in patients with initially severe disease or acute liver injury. Recovery differed by severity, with severe/critical patients showing greater magnitude of change of immune and inflammatory markers. Higher leucocytes, $CD8^+$ T cells, monocytes, and IL-6 in the severe/critical group at follow-up suggest ongoing low-grade immune remodeling. While higher ALT and $CD4^+$ T-cell counts in acute liver injury group indicate hepatic and immunological interaction, which is supported by correlations between immuno-inflammatory and hepatocellular injury markers. These findings support long-term monitoring of high-risk COVID-19 patients.

# FORMATION OF SARS-COV-2 NUCLEOCAPSID PROTEIN-RNA AGGREGATES IN CELL CULTURE CONDITIONS

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## Objectives

The SARS-CoV-2 Nucleocapsid (N) protein is the most abundantly produced protein during viral infection. Inside the host cell, it undergoes liquid-liquid phase separation upon interaction with viral genomic RNA, with the resulting condensates forming virus replication and viral particle assembly centers.

It has been shown that N protein is found outside the cell from early stages of infection and it's concentration in the blood correlates with severity of disease, making it a valuable diagnostic marker. The properties of liquid N protein-RNA condensates as they are found inside the cells during active infection are well characterized, however it is unknown how these N protein-RNA complexes form and behave upon entering the extracellular space and if they have any possible pathogenic outcomes. In order to use cell cultures as a reliable model for extracellular N protein pathogenicity, it is crucial to know what form such complexes take in culture conditions before observing cellular responses.

## Materials and Methods

Fluorescently labeled purified N protein and in-vitro transcribed SARS-CoV-2 genomic RNA fragments were mixed and incubated in different cell culture media and additives. The resulting aggregates were then imaged using brightfield and fluorescent microscopy.

## Results

N protein and RNA form solid protein-RNA aggregates in cell culture media. Labeled N protein and labeled RNA fluorescence signals colocalize, indicating co-precipitation. Similar aggregates form upon incubating N protein with non-specific RNA extracted from cells.

## Conclusions

N protein-RNA complexes form solid aggregates in cell culture media, rather than liquid condensates.

# SOLUBLE PD-1/PD-L1 IN PROSTATE CANCER: FROM IMMUNE CELL ASSOCIATIONS TO CLINICAL UTILITY

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**Objectives**

Introduction: Prostate cancer (PCa) develops within an immunosuppressive tumor–host environment involving multiple circulating immune-cell populations. Regulatory T cells (Tregs), natural killer (NK) cells, and distinct myeloid-derived suppressor cell (MDSC) subsets, including granulocytic MDSCs and monocytic MDSCs, may contribute to immune escape through different mechanisms. In parallel, the PD-1/PD-L1 axis represents a key checkpoint pathway regulating antitumor immune responses. Soluble PD-1 (sPD-1) and soluble PD-L1 (sPD-L1) are emerging liquid-biopsy biomarkers that may reflect systemic checkpoint activity and provide clinically relevant information for immune monitoring. However, their associations with specific circulating immune-cell subsets and clinical outcomes in PCa remain insufficiently defined.

Objectives:

This study aimed to evaluate the clinical and immunological relevance of soluble PD-1 and PD-L1 in prostate cancer by:

1. assessing associations between plasma sPD-1/sPD-L1 levels and circulating immune-cell subsets, including NK cells, Tregs, granulocytic MDSCs, and monocytic MDSCs;
2. investigating whether baseline sPD-1/sPD-L1 levels and their postoperative changes are associated with biochemical recurrence and progression-free survival;
3. exploring potential cellular contributors to circulating sPD-1/sPD-L1 profiles in PCa;
4. evaluating whether combinations of soluble checkpoint biomarkers with immune-cell subsets improve discrimination of clinically significant prostate cancer.

**Materials and Methods**

Peripheral blood from 88 patients with pT2–pT3 PCa was analyzed. Multiparametric flow cytometry quantified circulating immune-cell subsets. Plasma sPD-1 and sPD-L1 were measured by ELISA. Associations with immune-cell subsets and clinical outcomes, including biochemical recurrence and progression-free survival, were assessed. Diagnostic performance of single and combined markers was evaluated using ROC analyses.

**Results**

sPD-1 levels positively associated with NK cell frequencies, suggesting a link between soluble checkpoint regulation in PCa. Higher baseline sPD-L1 was associated with worse clinical outcomes, as patients with elevated preoperative sPD-L1 showed a tendency toward shorter postoperative progression-free survival during 30-month follow-up. Postoperative dynamics further separated patients into two biologically distinct groups: in one group, sPD-L1 significantly decreased after prostatectomy, supporting a tumor-derived contribution, whereas in another group sPD-L1 increased, suggesting alternative non-tumor or immune-cell-related sources. In patients with biochemical recurrence, preoperative sPD-L1 correlated strongly with Tregs and inversely with monocytic MDSC frequency, whereas no meaningful correlations were observed in patients with favorable postoperative courses. Importantly, combining sPD-L1 with granulocytic-MDSCs markedly improved discrimination of clinically significant PCa, reaching an AUC of 0.93 and 100% sensitivity, and outperforming combinations with monocytic MDSCs or Tregs. Overall, these findings suggest that sPD-L1 may reflect both tumor burden and systemic immunoregulatory activity in PCa.

**Conclusions**

Soluble PD-1/PD-L1 biomarkers reflect clinically relevant immune-cell associations in PCa and may support translational immune monitoring. Integration of sPD-L1 and sPD-1 with immune cells may improve risk stratification and help identify patients with clinically significant disease.

# SIZE AND STRUCTURE OF OLIGOMERIC PROTEINS SHAPE IMMUNOGENICITY AND PHAGOCYTE ACTIVATION

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**Objectives**

Oligomeric protein assemblies represent structurally distinct immunological stimuli that modulate both innate and adaptive immune responses. This study aimed to determine how the size, morphology, and origin of oligomeric proteins influence their immunogenicity and regulate phagocyte activation, with an emphasis on Fc receptor-mediated signalling due to immune complexes.

**Materials and Methods**

Defined Abeta(1-42) oligomers of varying sizes and recombinant viral oligomeric proteins, including spherical VP1-derived virus-like particles (VLPs) and structurally distinct nucleocapsid-like particles, were used. BALB/c mice were immunised to evaluate humoral responses and generate monoclonal antibodies. Murine macrophage cell lines (J774, BV-2) and primary macrophages were stimulated with oligomeric proteins and their immune complexes. Pro-inflammatory macrophage activation was assessed by analysing surface markers (F4/80, CD86, CD206, CD68) and cytokine production (TNF-alpha, IL-12/23, IL-10).

**Results**

Abeta oligomers exhibited pronounced size-dependent immunogenicity, with 1-2 nm species inducing the strongest IgG2a/IgG2b responses and revealing an immunodominant N-terminal epitope (aa 1-19). Larger oligomers and fibrillar forms were less effective in eliciting humoral responses. In macrophages, both host-derived and viral oligomeric proteins induced a cytokine response and polarisation, characterised by increased CD86 expression and elevated TNF-alpha and IL-23 secretion, consistent with pro-inflammatory macrophage activation. The magnitude and profile of activation depended on structural properties: smaller oligomers elicited stronger responses than fibrils, and spherical VLPs induced more pronounced activation than filamentous assemblies. VP1-derived VLPs triggered robust antibody responses with distinct immunogenicity profiles across polyomaviruses and demonstrated antigenic cross-reactivity, indicating shared structural epitopes and potential cross-immunity.

**Conclusions**

The data demonstrate that the immune effects of oligomeric proteins are governed by their structural properties. Small Abeta oligomers are the most immunogenic and define dominant B-cell epitopes, while both Abeta and viral oligomers drive pro-inflammatory macrophage activation, with profiles dependent on size and morphology. In parallel, VP1-derived VLPs induce strong, partially cross-reactive antibody responses, indicating conserved structural determinants of antigen recognition. Together, these findings show that oligomer structure links immunogenicity with phagocyte activation and shapes the overall immune response.

# IMPACT OF CODON OPTIMIZATION STRATEGY AND INDUCTION CONDITIONS ON HETEROLOGOUS SYNTHESIS OF TORQUE TENO VIRUS (TTV) ORF1ΔARG PROTEIN

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## Objectives

Torque teno virus (TTV) is a small, single stranded DNA virus belonging to the Anelloviridae family. It is a highly prevalent human virus that is considered as a non-pathogenic part of human virome, with no clear association with any specific diseases. Current evaluation of appropriate immunosuppressive therapy after organ transplantation relies on through levels of drugs in blood, which do not reliably predict infection and rejection risks, highlighting the need for improved monitoring tools. TTV load in blood is a potential immunosuppression level biomarker that may enhance current monitoring tools.
In this context, the objective of this study was to evaluate the impact of codon optimization strategy and induction conditions on the recombinant expression of the ORF1ΔArg protein of TTV, as a step towards the development of immunochemical detection approaches.

## Materials and Methods

ORF1ΔArg DNA sequence was ligated into pET-28a(+) plasmid under the control of T7lac promoter. The resulting construct was chemically transformed into competent Escherichia coli BL21 (DE3) cells. Expression of the recombinant protein fused to a 6xHis tag was induced in Luria-Bertani medium using different concentrations of isopropyl-D-1-thiogalactopyranoside (IPTG) and various temperatures. To improve recombinant protein production, codon optimization was performed using two bioinformatic tools (GenScript and Integrated DNA Technologies). Expression from optimized gene sequences was induced under the same conditions. Recombinant protein production was evaluated by Western blot targeting 6xHis tag.

## Results

Expression of recombinant viral protein from the original DNA sequence yielded no detectable protein at 37 °C temperature across all different IPTG concentrations. However, low levels were detected in samples at 25 °C with 0.1 and 0.05 mM IPTG. Based on these results, codon optimization was performed. Expression from optimized gene sequences resulted in detectable protein levels across all IPTG concentrations, with increased expression at 25 °C under all tested conditions. Among the optimized constructs, the sequence generated using GenScript tool showed relatively higher protein synthesis levels compared to that generated using Integrated DNA Technologies.

## Conclusions

Recombinant protein production from the original ORF1ΔArg DNA sequence under the tested induction conditions was insufficient for downstream applications. Codon optimization significantly improved expression levels. In particular, the sequence generated using the GenScript tool resulted in higher protein yields. Further optimization is required to establish conditions suitable for efficient production of this protein.

# DEFINING THE MELANOMA IMMUNE MICROENVIRONMENT THROUGH TRANSCRIPTOMIC PROFILING

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**1.** National Cancer Institute , **2.** National Cancer Institute

**Objectives**

Melanoma exhibits marked heterogeneity in tumour immune microenvironments, spanning immune-inflamed, immune-excluded, and immune-desert states. Characterising the transcriptional programmes associated with these phenotypes may reveal clinically relevant barriers to anti-tumour immunity and support biomarker-informed therapeutic strategies.

**Materials and Methods**

Bulk RNA-seq profiles from melanoma patients (n=38) were stratified into immune-desert (DES, n=17), immune-excluded (EXC, n=12), and immune-inflamed (INF, n=9) groups using a published two-step seven-gene classifier reflecting immune activity (CD2, CD53, IRF1, CD8B) and stromal patterns (COL5A2, TNFAIP6, INHBA). Tumour ecosystem features were quantified using the IOBR workflow, including ESTIMATE-derived immune and stromal scores, immune programme scoring, and cell-type deconvolution by CIBERSORT and EPIC. Differential expression analysis between phenotypes was followed by gene set enrichment analysis (GSEA) using MSigDB Hallmark and complementary pathway collections.

**Results**

DES tumours displayed globally reduced immune signatures and significantly lower immune and ESTIMATE scores compared with EXC and INF tumours. INF tumours were enriched for cytotoxic and antigen-presentation programmes, including T-cell-inflamed, TCR signalling, and NK/cytotoxic signatures. EXC tumours exhibited prominent stromal features, with increased fibroblast and cancer-associated fibroblast-associated signals, consistent with an immune-barrier phenotype. Hallmark GSEA demonstrated enrichment of interferon-gamma and interferon-alpha responses, inflammatory signalling pathways (TNF-NFkB, IL2-STAT5, IL6-JAK-STAT3), and allograft rejection in INF relative to DES. In contrast, EXC relative to INF was characterised by enrichment of epithelial-mesenchymal transition, TGF-beta signalling, angiogenesis, hypoxia, and coagulation pathways. DES tumours showed relative enrichment of proliferative and metabolic programmes compared with immune-active phenotypes. Immune checkpoint-associated scores were higher in INF and EXC than in DES, suggesting concurrent immune activation and adaptive immune suppression.

**Conclusions**

Transcriptome-derived immune phenotypes define distinct tumour ecosystem programmes in melanoma, corresponding to immune activation in INF, stromal and vascular exclusion in EXC, and immune paucity with proliferative wiring in DES. These findings support a translational framework for phenotype-informed immunotherapeutic strategies, including checkpoint blockade in INF, stromal/TGF-beta/angiogenesis-targeting combinations in EXC, and immune-priming approaches in DES.

# MONOCLONAL ANTIBODY-BASED DETECTION OF AMPC B-LACTAMASES IN ANTIBIOTIC-RESISTANT BACTERIAL ISOLATES

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**Objectives**

The increasing prevalence of antibiotic resistant bacteria poses a critical risk to human health globally. The emergence of class C β-lactamases (AmpCs) in Gram-negative bacteria is widely identified in healthcare settings, and these β-lactamases are increasingly being detected in livestock, wild and companion animals. The production of AmpCs confers high-level resistance to cephalosporins and β-lactam/β-lactamase inhibitor combinations. Consequently, accurate and easy-to-perform assays for the detection of AmpC-producing bacterial isolates are epidemiologically relevant, leading to more effective use of antibiotics and a comprehensive understanding of β-lactamase prevalence. Therefore, this study aimed to apply previously generated and characterized monoclonal antibodies (MAbs) raised against AmpCs for the detection of β-lactamases in bacterial isolates.

**Materials and Methods**

Recombinant CMY-34 was expressed in Escherichia coli and bacteriophage vB_EcoS_NBD2 tail tube protein gp39-derived nanotubes, as a scaffold displaying a highly conserved 17-amino acid peptide of AmpC β-lactamases, were produced in yeast, then used as an immunogens for MAb generation by hybridoma technology.

To prove MAb reactivity with AmpCs, the antibodies were tested with recombinant AmpC β-lactamases and AmpC-producing bacterial isolates. For this purpose, MAbs were applied in Western blot and sandwich-type assays, such as, lateral flow immunoassay (LFIA), and two-photon excitation (TPX) assay.

**Results**

One MAb panel raised against the CMY family (belongs to the AmpCs) of β-lactamases was selected and applied in LFIA and TPX assays. Another large collection of broadly reactive MAbs raised against a highly conserved 17-amino acid peptide of AmpC β-lactamases was employed for the detection of AmpCs using Western blot analysis. Both LFIA and TPX assays were able to detect all analyzed CMY-positive bacterial isolates producing CMY-2, CMY-4, CMY-6, CMY-16 and CMY-34 allelic variants. A panel of broadly reactive MAbs against AmpCs demonstrated cross-reactivity with all tested recombinant AmpC β-lactamases and β-lactamase-producing bacterial isolates in Western blot analysis.

**Conclusions**

In this study described novel MAbs recognize a wide range of AmpC enzymes and represent a promising tool for the immunodetection of antibiotic resistance determinants in bacterial isolates.

# ANTIBODY-BASED ANALYSIS DETECTS INFLAMMATION-LINKED CARBONIC ANHYDRASE IX EXPRESSION IN CERVICAL PATHOLOGY

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**Objectives**

Carbonic anhydrase IX (CA IX) is a hypoxia-induced enzyme increasingly recognized as a marker of tumor-associated microenvironmental changes, including inflammation. Accurate assessment of CA IX requires specific detection tools. This study aimed to develop and apply monoclonal antibodies (Mabs) targeting CA IX for biomarker analysis in cervical pathology, with particular focus on inflammation-associated alterations in cervical intraepithelial neoplasia (CIN).

**Materials and Methods**

MAbs against CA IX were generated and characterized for their specificity and ability to detect both membrane-associated and soluble forms. These antibody-based tools were applied to clinical samples from women with different grades of CIN. Vaginal microbiota status was evaluated, with emphasis on aerobic vaginitis as a marker of local inflammation. CA IX expression was assessed using immunological assays, and its association with histopathological findings and inflammatory status was analyzed.

**Results**

The developed MAbs enabled specific detection of CA IX in cell line models and clinical samples. Increased CA IX expression was associated with higher-grade cervical lesions. Importantly, elevated CA IX levels were significantly linked to inflammatory conditions, particularly aerobic vaginitis. Detection of soluble CA IX further supported its relevance as a biomarker reflecting both disease severity and inflammation-associated microenvironmental changes.

**Conclusions**

MAbs targeting CA IX provide reliable tools for investigating inflammation-associated changes in cervical pathology. The observed association between CA IX expression and inflammatory conditions highlights its potential as an integrative biomarker reflecting both hypoxia and local immune-related processes. These findings support the application of CA IX-targeting antibodies in translational studies focused on inflammation and disease progression.

# RNA LENGTH DEFINES SPECIES-SPECIFIC ANTIVIRAL RESPONSES AND HOST SUSCEPTIBILITY TO VIRAL INFECTION.

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**Objectives**

Innate immune recognition of viral RNA is a key determinant of host susceptibility to infection. However, how physical properties of viral RNA, such as length, shape immune activation across species remains unclear. We aimed to define how dsRNA length and 5'-end features regulate antiviral responses in different hosts and to determine whether species-specific differences in RNA sensing may contribute to altered susceptibility to RNA virus infections, including influenza.

**Materials and Methods**

Defined double-stranded RNA (dsRNA) molecules of varying lengths (short to ~1600 bp) and 5'-end modifications (5'-triphosphate or dephosphorylated) were generated by in vitro transcription. Human (A549), chicken (DF-1), and duck (CCL-141) cells were transfected with dsRNA. Innate immune activation was assessed using luciferase reporter assays, immunoblotting of antiviral pathway components, puromycin incorporation assays, and RNA integrity analysis. RNA-protein interactions were examined using dsRNA pull-down followed by mass spectrometry. Additionally, short RNA fractions derived from influenza-infected cells were tested for immunogenicity.

**Results**

Short 5'-triphosphorylated dsRNAs, including mini viral RNA (mvRNA)-like molecules, robustly induced interferon responses in human and duck cells but failed to do so in chicken cells. Removal of the 5'-triphosphate abolished immunogenicity, confirming dependence on canonical RNA sensing pathways. In contrast, long dsRNA triggered immune activation across all species, revealing a species-specific length threshold for dsRNA recognition, with chicken cells requiring substantially longer RNA (>~100 bp). Importantly, short RNA fractions derived from influenza-infected cells were immunogenic in human cells but not in chicken cells, demonstrating physiological relevance. PKR-dependent translational shutdown was induced by long dsRNA in all cell types, although with reduced intensity in duck cells. Activation of the OAS/RNase L pathway was observed exclusively in human cells. Proteomic analyses showed preferential binding of antiviral proteins, including PKR, to long dsRNA, with reduced enrichment in avian systems.

**Conclusions**

These findings identify RNA length as a critical determinant of innate immune activation and reveal a species-specific constraint in antiviral sensing. Chicken cells exhibit a functional deficit in detecting short viral RNAs, likely reflecting the absence of RIG-I-dependent pathways, which may impair early recognition of infection. This limitation provides a mechanistic explanation for differences in host susceptibility and highlights how tuning of innate immune thresholds can influence virus-host interactions and zoonotic risk.

# ACCUMULATION OF SMALL PERITONEAL MACROPHAGES AND DENDRITIC CELLS DURING TUMOR REJECTION IN A CONCOMITANT TUMOR IMMUNITY MODEL

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**Objectives**

Tumor-associated macrophages may have opposite effects on tumor growth, but their role in the antitumor immunity is not fully understood. In the DBA/2–SL2 concomitant tumor immunity model, secondary tumor growth is suppressed, indicating an important role for local immune responses. The aim of this study was to investigate the contribution of peritoneal macrophages and other immune cell populations to tumor control.

**Materials and Methods**

A DBA/2–SL2 concomitant tumor model was used, combining subcutaneous (SC) and intraperitoneal (IP) tumor challenges. Peritoneal immune cells were analyzed by flow cytometry, and macrophage function was examined using adoptive transfer and repeated intraperitoneal stimulation. Non-parametric statistical tests were used for data analysis.

**Results**

Secondary IP SL2 tumor rejection was associated with increased numbers of small peritoneal macrophages (SPM) and dendritic cells (DCs) in the peritoneal cavity, whereas primary IP tumors showed tumor cell proliferation. Tumor growth was associated with a decrease in the proportion of myeloid cells and B lymphocytes. Adoptive transfer of peritoneal macrophages alone did not prevent tumor growth, indicating that macrophages alone are unable to control tumor progression. Repeated IP injections of SL2 cells caused regression of 3 out of 5 SC tumors, suggesting a possible systemic immune antitumor effect.

**Conclusions**

The rejection of secondary IP SL2 tumors in DBA/2 mice was associated with a distinct peritoneal immune environment characterized by increased levels of SPM and DC. These findings suggest that small peritoneal macrophages and dendritic cells may be involved in tumor growth inhibition in concomitant immunity model model.

# THE ROLE OF TUMOR ANTIGEN PRESENTATION DEFECTS IN IMMUNOTHERAPY RESPONSE AND THEIR THERAPEUTIC TARGETING IN PRECLINICAL MODELS

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**Objectives**

Anticancer immunotherapy boosts patients' own immune system to recognize and eliminate tumor cells, and can provide long-term remission. However, low response rates to immunotherapy indicate prevalent resistance, highlighting the need to define its determinants and to develop rational combination treatment strategies. We investigated the impact of tumor Ag presentation mechanism (APM) status on responses to dendritic cell vaccination (DCV) and anti–PD-1 therapy, and tested whether cyclophosphamide (CY) can augment APM function and thereby improve chemoimmunotherapy efficacy in syngeneic C57BL/6 LLC1 and GL261 models.

**Materials and Methods**

C57BL/6 mice syngeneic LLC1 (Lewis lung carcinoma) and GL261 (glioma) tumors were assessed for APM status in vitro and tumor microenvironment in vivo (FC, qPCR). Autologous DCV were prepared from bone marrow (Lutz et al.) and loaded with tumor lysate (15 ?g/mL) and LPS (1 ?g/mL). Tumor-bearing mice received 3 doses of DCV (s.c., 1 × 106/100 µL PBS) or anti-PD-1 (i.p., 200 µg/100 µL PBS); tumor growth and induced immune response were monitored (FC, qPCR). CY effect on tumor APM was assessed in vitro (0,1-100 ?M) and in vivo (90-140 mg/kg) (FC, qPCR). The efficacy of combined CY, DCV and/or anti-PD-1 treatment was evaluated by tumor growth, APM status, and immune responses (FC, qPCR).

**Results**

Impaired tumor APM (LLC1 model) was associated with the absence of CD8+ T-cell response and resistance to DCV and anti-PD-1 therapy, whereas intact tumor APM (GL261 model) was associated with an elicited CD8+ T-cell response and sensitivity to immunotherapy. CY activated APM in LLC1 and GL261 cells in vitro and in GL261 tumors in vivo, together with induction of CD8+ T-cell response. Combined CY, DCV and anti-PD-1 treatment improved LLC1 and GL261 tumor control, but complete regression and immune memory were achieved only in mice with APM-intact tumors (GL261 model).

**Conclusions**

Tumor APM functionality determines antitumor immune response and sensitivity to DCV and anti-PD-1 therapy. Assessment of tumor APM status may have predictive value for patients' response to immunotherapy. CY enhances tumor APM function and synergizes with DCV and anti-PD-1. However, APM-impaired tumors may require additional interventions to achieve optimal CY-driven APM activation and the benefits of combination therapy.

# Angioedema

# CLINICAL CHARACTERISTICS OF HEREDITARY ANGIOEDEMA IN PATIENTS IN UKRAINE

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**Objectives**

To analyze the clinical characteristics of hereditary angioedema (HAE), including age at onset, diagnostic delay, and spectrum of symptoms in patients in Ukraine based on a survey.

**Materials and Methods**

A total of 48 patients with hereditary angioedema from Ukraine (29 females and 19 males), aged 6 to 73 years, were surveyed. Data were collected using a structured questionnaire and analyzed using descriptive statistical methods (n, %)

**Results**

The first symptoms of hereditary angioedema most commonly occurred before the age of 5 years (41.7%). Symptom onset was reported in 18.8% of patients at 6–10 years and in 16.7% at 11–15 years, whereas 8.3% of patients reported onset at 16–20 years and 8.3% after 20 years of age. Initial clinical manifestations were most frequently represented by edema of the upper extremities (47.9%), abdominal involvement (39.6%), and edema of the lower extremities (37.5%).

Despite the early onset of the disease, suspicion of HAE most frequently arose at the age of 21–30 years (27.7%). In the age groups 11–20 and 31–40 years, the rate was 21.3% each, while 12.8% of cases were suspected before the age of 10 years and between 41–50 years, and only 4.3% after 50 years.

According to the survey results, laboratory confirmation of HAE most commonly occurred at 21–30 years (32.6%). In 21.7% of cases, diagnosis was confirmed at 31–40 years, in 17.4% at 11–20 years, and in 15.2% at 41–50 years. Much less frequently, diagnosis was established before the age of 10 years and after 50 years (6.5% each).

The diagnostic delay from the onset of symptoms to confirmed diagnosis demonstrated substantial variability. The most frequent delay was 31–35 years (15.2%). In 13.0% of cases, diagnosis was established after 6–10 years or 21–25 years from symptom onset. In 10.9% of patients, the delay ranged between 2–5, 11–15, 16–20, or 36–40 years. A smaller proportion of patients had a delay of 25–30 years (6.5%), while in rare cases it reached 41–45 years (2.2%) or 51–55 years (2.2%). Only 4.3% of patients were diagnosed within the first year after symptom onset.

The most clinically significant symptoms reported during the disease course were abdominal manifestations (68.8%), upper extremity edema (47.9%), lower extremity edema (45.8%), and laryngeal edema (31.3%).

**Conclusions**

Hereditary angioedema is characterized by early onset, typically in childhood; however, in most patients, diagnosis is established only in young or middle adulthood. A substantial diagnostic delay was identified, reaching several decades in a considerable proportion of patients. The most common clinical manifestations include abdominal and peripheral edema, while laryngeal involvement occurs in nearly one-third of patients and represents a potentially life-threatening condition. These findings highlight the need to improve physician awareness and promote earlier recognition of hereditary angioedema.

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