Network Analysis of the Serum Proteome in a Prospective Cohort of Immune Checkpoint Inhibitor Recipients Highlights Interferon-γ as a Pivotal Mediator of Immune-Related Adverse Events

Authors: *Joe Berry,[1,2] Kristian Williams,[1,2] Abigail Gault,[1,3] Linda Hogarth,[3] Abbie Degnan,[1,2] Julie Diboll,[1,2] Imogen Wilson,[1,2] Amy E. Anderson,[1,2] Ruth Plummer,[1,3] Arthur Pratt[1,2]

1. Translational and Clinical Research Institute, Newcastle University, UK
2. National Institute for Health and Care Research, Newcastle Biomedical Research Centre, UK
3. Northern Centre for Cancer Care, The Newcastle upon Tyne Hospitals NHS Foundation Trust, UK

*Correspondence to Joe.Berry@newcastle.ac.uk

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BACKGROUND AND AIMS

Immune checkpoint inhibitors (ICI) have transformed cancer therapy but carry a significant risk of immune-related adverse events (irAE), which may be sustained and life-altering.[1] Among these, inflammatory arthritis develops in ≥6% of ICI-treated patients, representing one of the more common irAEs. Understanding the mechanisms driving irAEs is essential to optimise irAE management without compromising the anti-tumour response. Moreover, given probable shared disease pathways, understanding their mechanisms may provide vital insights into the development of 'spontaneous' immune-mediated inflammatory diseases (IMID), including inflammatory arthritis.

The aim of this study was to examine the pathobiology underlying irAE development through integrated cytometric, proteomic, and network-based analysis.[2]

MATERIALS AND METHODS

Fifty-seven adults with a cancer diagnosis receiving ICIs as part of routine care and enrolled into the MEDALLION cohort were included. Participants were followed longitudinally, with serial blood samples collected during ICI infusions from baseline until approximately 10 months' follow-up or the visit immediately preceding an irAE.

Two hundred and fifty unique serum proteins were profiled using the NULISAseq (Alamar Biosciences, Silicon Valley, California, USA) inflammation panel and normalised using the standard NULISAseq workflow, including internal- and inter-plate control normalisation, $\log_2$ transformation, and final intensity scaling to produce plate-harmonised values. In parallel, serially obtained peripheral blood mononuclear cells were analysed using multiparameter flow cytometry, with a focus on T cell subsets and activation status.

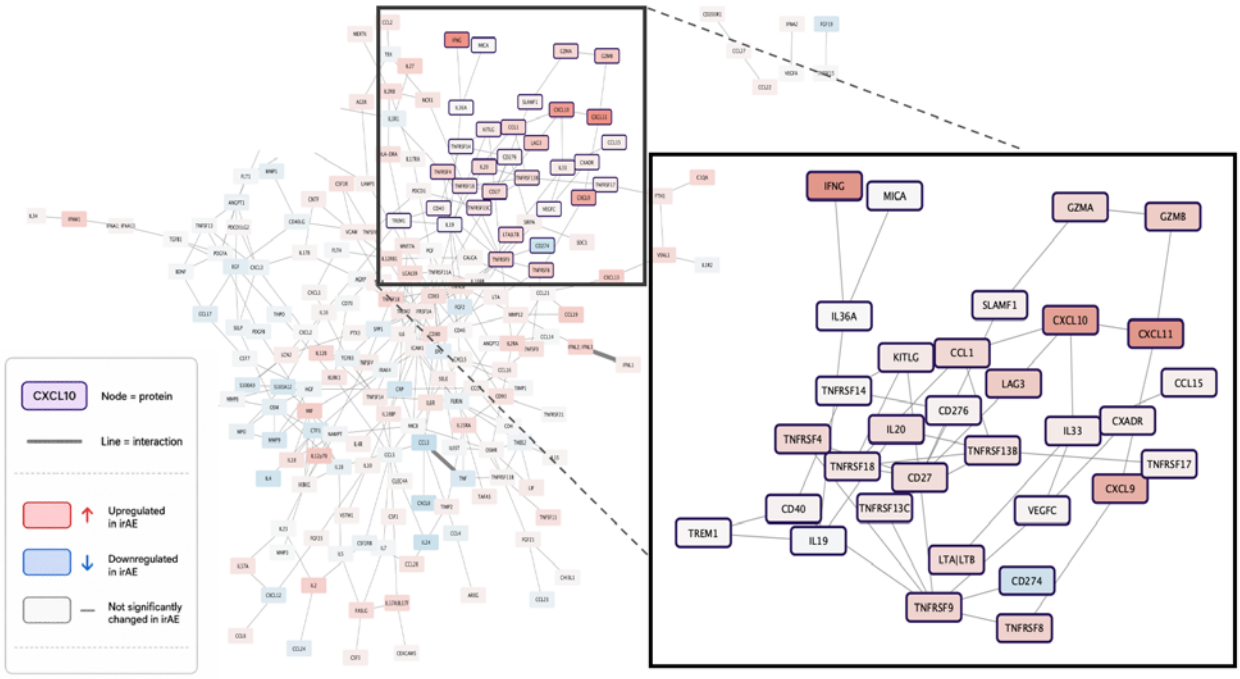
To explore protein–protein relationships, a baseline network was inferred using the ARACNE algorithm, which uses mutual information to identify statistically dependent relationships between proteins, including both linear and non-linear associations, while removing indirect interactions. Differential protein expression was overlaid onto this network to identify subnetworks of coordinated alteration

in participants who developed irAEs. Longitudinal changes in protein relationships were further examined using Differential Gene Correlation Analysis (DGCA).

RESULTS

The cohort comprised 49 (86%) participants with malignant melanoma, three (5%) with lung adenocarcinoma, and five (9%) with mesothelioma. Thirty-one participants (54%) received combination therapy, including anti-CTLA-4 at initiation alongside anti-PD-1 or anti-PD-L1 agents. Combination therapy recipients were significantly more likely than those receiving monotherapy to develop ≥1 irAE, and a higher proportion of participants with irAEs were classified as ICI responders (complete, partial, or stable disease) by the end of follow-up. Baseline demographics and clinical characteristics did not otherwise differ significantly between groups.

Figure 1: Baseline ARACNE protein network.



Networks were visualised in Cytoscape (The Cytoscape Consortium, San Diego, California, USA). Nodes represent proteins; edges denote direct associations inferred from baseline expression. Node colour indicates delta expression (pre-irAE – baseline) between participants that developed an irAE and those that did not (red: higher in irAE; blue: lower). IFN-γ and its inducible chemokines (CXCL9, CXCL10, CXCL11) were upregulated in irAE participants and clustered within the same baseline module.

AKT1: AKT serine/threonine kinase 1; ARACNE: Algorithm for the Reconstruction of Accurate Cellular Networks; CCL: C-C motif chemokine ligand; CD: cluster of differentiation; CXADR: coxsackievirus and adenovirus receptor; CXCL: C-X-C motif chemokine ligand; FLT1: fms-related receptor tyrosine kinase 1; GZM: granzyme; IFNG: interferon gamma; IL36A: interleukin 36 alpha; irAE: immune-related adverse event; KITLG: KIT ligand (stem cell factor); LAG3: lymphocyte activation gene 3; MICA: major histocompatibility complex class I polypeptide-related sequence A; SLAMF1: signalling lymphocytic activation molecule family member 1; TLR1: toll-like receptor 1; TNFRSF4: tumour necrosis factor receptor superfamily member; TREM1: triggering receptor expressed on myeloid cells 1; VEGFC: vascular endothelial growth factor C; VSNL1: visinin-like protein 1.

The exploratory ARACNE network highlighted a highly interconnected subnetwork enriched for interferon-gamma (IFN-γ)-related proteins, including IFN-γ, CXCL9, CXCL10, CXCL11, granzyme A, granzyme B, and LAG3. Proteomic analysis demonstrated increased expression of proteins within this subnetwork, particularly IFN-γ and its associated chemokines, in participants who subsequently developed irAEs (Figure 1). DGCA confirmed persistence of the baseline protein relationships and identified multiple protein pairs exhibiting coordinated longitudinal change, suggesting concerted upregulation of the IFN-γ-centred network prior to clinical irAE manifestation.

Alongside the proteomic findings, cytometric analysis showed a less-activated baseline $CD8^+$ memory T cell phenotype in participants who developed irAEs, followed by markedly greater post-ICI induction, as evidenced by increased CD38 expression on $CD45RO^+CD8^+$ T cells.

CONCLUSION

The authors' longitudinal proteomic analysis demonstrates coordinated upregulation of peripheral IFN-γ and IFN-γ-induced chemokines prior to the development of irAEs. In parallel, participants developing irAEs showed a more robust activation of memory $CD8^+$ T cells following checkpoint inhibition. These findings identify potential biomarkers that precede irAEs and support a model in which checkpoint inhibition amplifies IFN-γ-driven CXCL9/10/11–CXCR3 signalling, promoting the recruitment of activated T cells and establishing a positive feedback loop that drives peripheral tissue inflammation. Furthermore, these findings provide insight into immune pathways that may be shared between irAEs and spontaneous IMIDs, including inflammatory arthritis.

References

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