

# Serum IgG responses to melanoma-associated peptides do not distinguish birdshot uveitis: the MACAU study

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## Short Report

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# Abstract

## Background

Birdshot uveitis (BU) is strongly associated with HLA-A29, but its antigenic trigger remains unknown. Reports linking melanoma-directed immunity to birdshot-like inflammation raise the possibility that patients with BU share a circulating antibody response to melanoma-associated antigens.

## Methods

In MACAU-1, pooled sera from birdshot, intermediate and sarcoid uveitis and healthy control groups were screened on prefabricated melanoma, oncoviral and autoimmune peptide arrays. Peptides with strong IgG binding or an apparent between-group difference were transferred to a custom array. MACAU-2 tested these candidates, together with 44 overlapping mycobacterial ESAT-6-like protein EsxB peptides, in individual sera from 50 participants: BU (n = 15), intermediate uveitis (n = 10), sarcoid uveitis (n = 15) and healthy controls (n = 10).

## Results

Serum IgG from all four study groups bound melanoma-associated peptides, but no peptide or melanoma-associated parent protein distinguished BU from the other uveitis groups or healthy controls after correction for multiple testing. The strongest nominal differences generally reflected lower, not higher, reactivity in BU. Analyses of the autoimmune, oncoviral and ESAT-6-like protein EsxB candidates likewise yielded no disease-specific serum IgG signature.

## Conclusions

BU was not associated with a shared serum IgG response to the melanoma-associated linear peptides tested. This argues against a clinically useful circulating anti-melanoma antibody signature, but does not exclude melanocyte-derived antigens as targets of HLA-A29-restricted cellular immunity.

## Background

Birdshot uveitis (BU) is the most strongly HLA-associated human disease: all patients carry HLA-A29, yet the allele is common in the general population and is insufficient to cause disease [1]. Associations with ERAP1 and ERAP2 further implicate intracellular peptide trimming and HLA class I-restricted T-cell recognition [2–4]. The antigen that initiates or sustains this response remains unknown [5].

Melanoma-associated immunity offers a clinically coherent hypothesis. BU primarily affects the melanocyte-rich choroid, and birdshot-like inflammation has been reported during immune checkpoint

inhibitor treatment for melanoma [6]. Although such drug-associated, HLA-A29-negative disease is not equivalent to classical BU, it shows that antitumour immunity can cross-react with ocular tissue [7, 8]. A conference abstract reported anti-melanoma serum immunoreactivity in five patients with BU [9]. These observations prompted us to ask whether a larger BU cohort carries a reproducible serum IgG response to melanoma-associated peptides.

MACAU used a two-stage design: broad pooled screening for candidate reduction, followed by individual testing. Intermediate and sarcoid uveitis served as clinically distinct inflammatory comparators, while oncoviral, autoimmune and mycobacterial candidates allowed related antigenic hypotheses to be explored without changing the principal question: does BU show a distinctive circulating IgG response to melanoma-associated antigens?

Although BU is considered predominantly T-cell mediated, a reproducible serum IgG response could provide an accessible marker of shared antigen exposure or melanoma-related cross-reactive immunity, without necessarily implying that antibodies are the primary disease mechanism.

## Methods

MACAU was a single-centre exploratory study at University Hospitals Leuven. Adults with BU, idiopathic intermediate uveitis or sarcoid uveitis and healthy controls were recruited between 10 November 2023 and 27 June 2025. Uveitis was classified according to Standardization of Uveitis Nomenclature terminology. All patients with BU fulfilled accepted diagnostic criteria, including HLA-A29 positivity. Key participant characteristics are reported in Table 1. The birdshot group had a higher mean age and a greater proportion of participants receiving systemic immunomodulatory treatment.

In MACAU-1, equal-volume sera from five participants per group were pooled. PEPperPRINT GmbH (Heidelberg, Germany) screened one pool per group on three prefabricated peptide microarrays covering melanoma-associated, oncoviral and autoimmune peptides. Arrays were processed and scanned by PEPperPRINT, with initial analysis using PEPperMAP software. Peptides producing the strongest IgG signals or an apparent difference between groups were selected for the MACAU-2 custom array. MACAU-1 was used solely to reduce the candidate set: pooling provided no information on individual responses. The 20 donors contributing to the pools were therefore retested individually in MACAU-2.

The custom array contained 768 experimental peptide spots—440 autoimmune, 132 melanoma-associated, 152 oncoviral and 44 overlapping ESAT-6-like protein EsxB peptides—and three control spots. After duplicate peptide sequences were collapsed, these represented 765 unique experimental peptide sequences. Individual sera from 50 participants were tested: BU ( $n = 15$ ), intermediate uveitis ( $n = 10$ ), sarcoid uveitis ( $n = 15$ ) and healthy controls ( $n = 10$ ). PEPperPRINT manufactured and processed the arrays, scanned the slides, corrected the background signal and performed the initial analyses. The complete processed dataset was subsequently independently reanalysed by the UMC Utrecht team.

Because many signals remained at background level, detection above local background was the primary outcome and signal intensity was supportive. Groups were compared at peptide level and after grouping peptides by parent protein or sequence similarity, with correction for multiple testing. Full analytical details are provided in the Supplementary Methods.

## Results

All 50 sera yielded evaluable arrays. Overall peptide detection and IgG signal strength were comparable across the four groups ( $p = 0.39$  and  $p = 0.15$ , respectively), indicating no general increase or decrease in circulating peptide-reactive IgG in any uveitis phenotype.

The melanoma panel contained 132 peptides derived from 19 melanoma-associated proteins, including tyrosinase, melanoma-associated antigen (MAGE) family members, preferentially expressed antigen in melanoma (PRAME), p53, MYC and melanoma cell adhesion molecule. After quality and detection filtering, 83 peptides were assessed by intensity and 82 by seropositivity. Normalized IgG reactivity to melanoma-associated peptides across individual sera is shown in Supplementary Figure S1. No individual melanoma-associated peptide distinguished BU from intermediate uveitis, sarcoid uveitis or healthy controls after multiple-testing correction. The same was true when responses were combined by their 18 represented parent proteins. The strongest unadjusted differences generally showed lower reactivity in BU, providing no support for a shared increase in anti-melanoma IgG.

The broader analysis led to the same conclusion. No autoimmune, oncoviral or ESAT-6-like protein EsxB peptide, parent protein or sequence-related peptide cluster produced a disease-specific signature after correction for multiple testing. An analysis designed to detect weaker signals distributed across the full IgG repertoire was also negative. The initial PEPperPRINT analyses and our reanalysis yielded concordant conclusions.

## Discussion

In this individual-level follow-up study, serum IgG against melanoma-associated peptides did not distinguish BU from other uveitis phenotypes or healthy controls. We did not confirm a distinctive anti-melanoma serum IgG response in BU using this linear peptide panel, and the tested panel has no demonstrated value as a serum biomarker for the disease.

This negative result does not invalidate a melanocyte-antigen hypothesis. Serum antibodies against immobilised linear peptides interrogate a different immune compartment from the leading BU model, in which ERAP-dependent peptide processing, HLA-A29 presentation and CD8 + T-cell recognition are central. A melanoma-associated or melanocyte-derived protein could still supply the relevant HLA-A29-bound peptide without inducing a detectable circulating IgG response. Similarly, immune checkpoint inhibitor-associated birdshot-like inflammation supports a possible antitumour–ocular cross-reaction, but does not establish a humoral mechanism.

The negative findings in the comparator panels should be interpreted just as narrowly. They do not exclude an EBV-related mechanism in intermediate uveitis [10, 11] or a mycobacterial contribution to sarcoidosis [12], because both hypotheses may depend more on compartmentalised or T-cell responses than on serum IgG. Future studies should therefore prioritise HLA-A29 immunopeptidomics, peptide–HLA binding, and antigen-specific CD8 + T-cell assays, potentially combined with T-cell receptor sequencing, rather than further untargeted serum antibody screening.

The principal limitations are the modest cohort size, lack of matching for age and sex, variation in disease activity and systemic immunomodulatory treatment, and restriction of the array to selected linear peptides. Because the donors contributing to the MACAU-1 pools were retested in MACAU-2, the individual analysis did not constitute an independent validation cohort. However, this overlap would be expected to favour persistence of signals selected during pooled screening rather than explain their absence in the individual analysis. Conformational, post-translationally modified, rare or exclusively intraocular responses may have been missed. Accordingly, MACAU did not identify a strong and shared serum IgG signature within this panel. Smaller, heterogeneous or treatment-dependent differences cannot be excluded. These findings also do not exclude antigen-specific cellular immunity in BU.

Table 1  
participant characteristics

| Characteristic                                                  | Birdshot uveitis (n = 15) | Intermediate uveitis (n = 10) | Sarcoid uveitis (n = 15) | Healthy controls (n = 10) |
|-----------------------------------------------------------------|---------------------------|-------------------------------|--------------------------|---------------------------|
| Age, years, mean ± SD                                           | 61.9 ± 7.0                | 45.6 ± 11.5                   | 52.5 ± 14.1              | 42.6 ± 16.4               |
| Female sex, n (%)                                               | 10 (66.7)                 | 3 (30.0)                      | 8 (53.3)                 | 5 (50.0)                  |
| Disease duration, years, mean ± SD                              | 7.2 ± 4.7                 | 5.6 ± 4.8                     | 5.4 ± 3.9                | Not applicable            |
| Current systemic immunomodulatory treatment, n (%) <sup>1</sup> | 12 (80.0)                 | 5 (50.0)                      | 8 (53.3)                 | Not applicable            |

<sup>1</sup> Includes current systemic corticosteroid, conventional immunomodulatory or biologic treatment. Previous treatment was not counted. Disease duration was calculated from the recorded diagnosis date to serum sampling.

## Abbreviations

- BU
- birdshot uveitis
- ERAP
- endoplasmic-reticulum aminopeptidase
- HLA

human leukocyte antigen  
MACAU  
Micro-Array Chip Analysis in Uveitis  
SUN  
Standardization of Uveitis Nomenclature.

## Declarations

**Ethics approval and consent to participate:** The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee Research UZ/KU Leuven: MACAU-1 (S67885; Belgian registration B3222022001249; favourable opinion 23 October 2023) and MACAU-2 (S68975; Belgian registration B3222024001571; initial favourable opinion 19 December 2024). All participants provided written informed consent before study procedures.

**Consent for publication:** Not applicable; no identifiable individual participant data are presented.

**Availability of data and materials:** The data supporting the findings of this study are available from the corresponding author on reasonable request, subject to applicable institutional and data-protection requirements.

**Competing interests:** The authors declare no competing interests. PEPperPRINT GmbH acted as a commercial laboratory service provider and performed array manufacture, processing, scanning, background correction and initial analysis; it had no authorship role in this manuscript.

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**Authors' contributions:** PPS conceived and coordinated the study, recruited participants, collected and analysed the data, interpreted the results and drafted the manuscript. JJ supervised participant recruitment and study conduct. KdV contributed to data analysis and interpretation. JJWK performed the independent statistical analysis and contributed to interpretation of the results. SS contributed to study conception, sample processing, data analysis and interpretation, and supervised the laboratory work. All authors critically revised the manuscript and read and approved the final version.

**Use of Generative AI:** Generative AI tools (ChatGPT, OpenAI) were used during manuscript preparation for language editing and structural suggestions. All scientific content, numerical results and interpretations were critically reviewed and verified by the authors, who take full responsibility for the final manuscript.

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## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMethods.docx](#)
- [SupplementaryFigure1MelanomaPeptideHeatmap.pdf](#)