Joint Research Management Office (JRMO) Research Protocol for Research Studies

Full Title	Investigating pro-inflammatory B-lymphocyte responses in nasal polyps to interleukin-5
Short Title	BLYNI5
Sponsor	Queen Mary University of London (Queen Mary) Contact person: Mays Jawad R & D Operations Manager Queen Mary University of London 69 – 89 Mile End Road London, E1 4UJ Phone: 020 7882 7275/6574 Email: research.governance@qmul.ac.uk
IRAS Number ***Integrated Research Application System***	**332970**
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REC Reference ***Research Ethics Committee***	
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1. Glossary

AE	Adverse Event
CI	Chief Investigator
CRF	Case Report Form
GCP	Good Clinical Practice
IL-	Interleukin-
JRMO	Joint Research Management Office
Participant	An individual who takes part in a clinical trial
PI	Principal Investigator
PIS	Participant Information Sheet
REC	Research Ethics Committee
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
WP	Work Package

2. Signature page

CI Agreement

The study, as detailed within this Research Protocol, will be conducted in accordance with the principles of Good Clinical Practice (GCP), the UK Policy Framework for Health and Social Care Research, and the Declaration of Helsinki and any other applicable regulations. I agree to take responsibility for the statistical analysis and oversight of this study.

CI Name: ___Dr Louisa James_____

Signature: ___ _____

Date: __02/01/2024_____

Louisa James

Digitally signed by Louisa James
Date: 2024.03.08 13:41:42 Z

3. Summary and synopsis

Short title	BLYNI5
Methodology	Laboratory-based research study using nasal tissue samples from patients with nasal polyps not on biologics (n=16), with polyps on anti-IL-5 pathway biologics (n=6) and control patients without polyposis (n=6)
Objectives / aims	To assess the effect of IL-5 treatment/blockade on the BCR (immunoglobulin) repertoire of tissue resident airway B cells
Number of participants	22-28 patients depending on number of patients whose samples are used in both WP1 and WP2 WP1: 10 patients WP2: 18 patients
Inclusion and exclusion criteria	Major Inclusion Criteria: Patients (Aged 18 years and over) undergoing resection of nasal tissue for clinical indications (e.g. polypectomy, turbinectomy, septoplasty, tonsillectomy). Able to give consent. Exclusion Criteria: Inability to give consent Previous Rituximab treatment (ever) Chemotherapy within preceding 6 months Cystic fibrosis Known current COVID infection/TB infection
Study duration	5 years

4. Introduction

4.1. Background

The overlapping conditions of severe asthma, nasal polyps and EGPA are all characterised by chronic eosinophilic airways inflammation. This has led to the successful development of monoclonal antibody treatments that target the primary cytokine for eosinophilic inflammation, interleukin-5 (IL-5). However the receptor for IL-5 is also expressed by other cell types and of note IL-5 was first named as a 'B cell growth factor'. Anti-IL-5 monoclonal antibody therapy may therefore have clinical action through effects on other cell types other than just eosinophils and potentially much of the beneficial effect of blocking IL-5 may be due to the effects on these other cell types. This hypothesis is supported by the excellent response of nasal polyps in clinical trials to anti-IL-5 Mepolizumab but poor clinical response to the eosinophil-depleting Dexpramipexole [Laidlaw et al. 2019], and relatively weaker effect of eosinophil-depleting Benralizumab than other biologics in nasal polyp disease [Cai et al. 2022]. The expression of IL-5Ra on other cell types than just eosinophils may also influence safety considerations for Benralizumab (that targets IL-5Ra expressing cells for cell-mediated killing) as compared to biologics that block the cytokine itself. We therefore ask the question – Do IL-5 expressing B cells rather than eosinophils drive chronic rhinosinusitis with nasal polyps and associated eosinophilic airway diseases [Kariyawasam and James, 2021]?

This is an important question as it would have an impact on the current debate over which of suppression of IL-5 (as seen with Mepolizumab) or depletion of IL-5R expressing cells (Benralizumab) is more effective and which safer – in particular depletion of IL-5R expression B cells may theoretically predispose to specific antibody deficiencies and thereby infection, or other perturbations of healthy human immunity.

Activation of B cells outside of secondary lymphoid organ germinal centres occurs in peripheral sites such as nasal polyps, under mechanisms controlled by the local peripheral tissue environment [Feldman et al. 2017]. Importantly extrafollicular B cell activation can bypass usual negative selection pressures and thereby is potentially more prone to lead to auto-antibody formation. B cells can respond to IL-5 in terms of differentiation and antibody class switching [Takatsu et al. 1998]. Plasma cells, fully differentiated B cells, in nasal polyps express IL-5Ra and respond to IL-5 [Bucheit et al. 2020]. In the MATERIAL study Mepolizumab enhanced bronchial secretory IgA production [Sabogal Pineros et al. 2019]. In contrast, IL-5Ra knock-out mice show selective immunoglobulin subclass deficiencies [Yoshida et al. 1996]. Whether IL-5 blocking and IL-5Ra depleting therapies will have the same effect on B cells is uncertain. Immunological therapies can affect B cell immunoglobulin repertoires in a manner consistent with clinical activity [James et al. 2012].

4.2. Rationale

This research will determine the actions of IL-5 and its blockade on tissue resident B cells in nasal polyps. Specifically, we will examine whether IL-5 contributes to class-switching, influences B cell survival and maturation, influences the expression of particular antibody subclasses.

4.3. Risks / benefits

This study will examine antibody-producing B cells in nasal tissue from patients undergoing surgery as part of their normal clinical care. The tissue samples would normally be discarded following surgery. Therefore, participation in this study does not carry additional risks. There are no direct benefits for the participants.

5. Study objectives

This is a laboratory-based study that aims to assess the effect of IL-5 treatment/blockade on the BCR (immunoglobulin) repertoire of tissue resident B cells. The primary objective is to assess the effect of IL-5 treatment / blockade on the BCR (immunoglobulin) repertoire of tissue resident airway B-cells, in particular on relative expression of different immunoglobulin classes and subclasses, in particular examining:

(i) **The relative expression across the tissue BCR repertoire of different immunoglobulin classes and subclasses in the presence/absence of IL-5 pathway blockade.** The relative expression of IgE (pro-allergic), IgA (mucosal defense) and IgG4 (pro-tolerogenic in allergy but also increased in persistent nasal polyps and EGPA) immunoglobulins determines the effectiveness of antibody responses in health and disease.

a. We will examine whether relative expression of different immunoglobulin classes/subclasses is perturbed by IL-5 pathway blockade using BCR repertoire analysis.
b. We will analyse immunoglobulin VDJ hypermutation as a measure of affinity maturation of resident B cells, and whether this is affected by IL-5/blockade.

(ii) **Effects on the BCR repertoires specific to relevant target antigens in the presence/absence of IL-5 pathway blockade.** B cell responses to specific pathogen antigens (e.g. Staphylococcus aureus) and auto-antigens (e.g. myeloperoxidase) are hypothesised to be of significant importance in the pathology of nasal polyps.

a. We will examine by BCR repertoire analysis the relative expression of immunoglobulin to target antigens of different classes/subclasses and whether this is perturbed by IL-5 / blockade of the IL-5 pathway.

Secondary objectives will include: Comparing BCR repertoires for convergent clonotypes evident in blood (from IDEA project) and other available BCR libraries, to assess for potential pathological clonotypes in eosinophilic airway inflammation. As single-cell sequencing will sequence both immunoglobulin heavy and light chains, in future experimental work recombinant antibodies could be generated from the sequences to further study antigenic targets of cloned antibody. If clonotypes are evident then to examine whether B cells expressing those clonotypes also express IL-5Ra and respond to IL-5.

These objectives will be studied in two work packages:

(WP1) In Vitro WP: Nasal polyp tissue from donors not on biologic therapy will be cultured with/without non-selective / target-antigen stimulation with/without recombinant IL-5 and/or blocking antibodies to IL-5 (mepolizumab in culture). BCR repertoires will be analysed by bulk RNA sequencing. Findings from BCR repertoire sequencing will be confirmed by ELISA/FACS for different antibody classes and subclasses secreted by B cell in cell culture. This WP will examine the effects of the IL-5 pathway in vitro and help optimise experiments for WP2.

(WP2) Ex Vivo WP: Nasal tissue from patients without nasal polyps, from patients with nasal polyps not on biologic therapy, and from patients with nasal polyps on biologic therapy will be analysed ex vivo for BCR repertoire by single-cell RNA sequencing. Samples from the donors for the ex-vivo WP may additionally be examined with in vitro cultures as per WP1. This WP will examine the effects of the IL-5 pathway in vivo.

5.1. Research Questions

What are the actions of IL-5 and its blockade on tissue resident B cells in nasal polyps? Questions that follow include:

- Does IL-5 contribute to class-switching - whether class of immunoglobulin secreted differs in the presence of IL-5 / blockade?

- Does IL-5 differentially contribute to survival of B cells expressing B cell receptors (immunoglobulins) of different classes and subclasses?

- Does IL-5 / blockade affect affinity maturation of B cells, necessary for optimal antibody responses to new and evolving pathogens?

- Are there clonotypes evident in nasal tissue of patients with nasal polyps selective for eosinophilic disease?

- Are there clonotypes evident in nasal tissue of BCR specific for putative auto-antigens (extending findings of current GSK-funded IDEA study that will be analysing BCR repertoires in blood from characterised patients)? - Do B cells expressing these clonotypes express IL-5Ra and respond to IL-5?

6. Study population

WP1: We will recruit the following:

- Patients undergoing nasal polyp resections and tonsillectomy (n=10)

WP2: We will recruit to the following groups:

- Nasal polyps not on biologics (n=6)
- Nasal polyps on anti-IL-5(R) biologics (n=6)
- Control patients without nasal polyposis (n=6)

We will use purposive sampling in our recruitment of the nasal polyps not-on-biologics and control patients groups to attempt to match patient characteristics (i.e. asthma status, age and gender) to the patients with polyps on biologics, which is predicted to be the more difficult group to recruit. Control patients will predominantly be patients undergoing nasal surgery for deviated nasal septa (structural rather than immunological pathology).

Research tissue from a single patient may be used for both WP1 and WP2.

6.1. Inclusion criteria (both WP)

- Patients undergoing resection of nasal tissue for clinical indications (e.g. polypectomy, turbinectomy, septoplasty, tonsilectomy).
- Able to give consent.
- Age 18 years and over

6.2. Exclusion criteria (both WP)

- Inability to give consent
- Previous Rituximab treatment (ever)
- Chemotherapy with preceding 6 months
- Cystic fibrosis
- Pregnancy
- Breast feeding
- Known current COVID infection/TB infection

7. Study design

This is a single visit study:

Study Procedure	**Visit 1**
Informed Consent	X
Confirmation meets Inclusion/Exclusion Criteria	X
Demographics	X
Medical History	X
Collection in saline of residual tissue removed during clinical procedure	X
Sample Processing	X (Processing as per WP)

Table 1: Schedule of Events

(WP1) In Vitro WP:

For *in vitro* experiments, airway tissue will be collected from patients undergoing clinically-indicated resection of nasal polyps and tonsils, dissociated, and B cells separated by magnetic or flow-cytometric isolation. The B cells will then be cultured *in vitro* both with and without stimulation, with and without the presence/absence of recombinant IL-5, Mepolizumab anti-IL-5 antibody, and relevant permutations of cell culture conditions:

Stimulations: unstimulated, anti-CD40 pan B cell stimulation, Staphylococcal enterotoxins (SEA, SEB, SED, SEE), Covid antigens

B cell phenotype after culture will be examined by flow cytometry, secreted antibody measurements by ELISA (including measurement of different subclasses), and bulk antibody repertoire sequencing conducted with BCR-specific primers.

Peripheral blood (20 mls) will also be collected as on optional additional activity pre-operatively on the day of surgery. Serum and peripheral blood mononuclear cells will be separated on day of collection. Serum will be stored frozen for future antibody and circulating cytokine ELISA experiments. Peripheral blood mononuclear cells will be stored for future use in analysis of peripheral blood B cell BCR transcriptome and B cell stimulation assays, to probe mechanistic aspects of the research question.

At selected sites we will optionally sample immune mediators (e.g. cytokines) in nasal secretions by nasoabsorption pre-operatively. Secretions will be collected by centrifugation of sponges through a microfilter and frozen at -80°C. Immune mediators (in particular IL-5) will be measured using multiplexed immunoassays (e.g. Luminex) and compared to BCR transcriptome.

These experiments will be used to refine methodology for WP2.

(WP2) Ex Vivo WP:

Nasal tissue will be collected from patients with nasal polyps not on biologics (n=6), with polyps on anti-IL-5(R) biologics (n=6) and control patients without nasal polyposis (n=6) undergoing clinically-indicated nasal surgery. Samples will be dissociated, and cells labelled with oligonucleotide-barcoded antibodies to CD20 and IL-5Ra. Single-cell B cell receptor (BCR) sequencing will be undertaken in the QMUL Core Genomics facility, and BCR repertoire (i.e. immunoglobulin repertoire) analysed to identify the immunoglobulin class, subclass and antibody sequence of tissue B cells from the three patient groups, stratified by IL-5Ra expression. Complementary bulk RNA sequencing with BCR-specific primers will be conducted to increase the depth of sequencing and enable further comparisons between groups.

These samples may additionally be examined by bulk RNA BCR sequencing after *in vitro* stimulation as per WP1. Optional collection of blood and nasoabsorption samples will be conducted at selected sites similar to WP1.

8. Study procedures

Invitation to Participate:

Invitation to Participate will be by members of the clinical team who will provide potential participants with a copy of the Participant Information Sheet (PIS) in advance of their scheduled surgery.

Patients will be identified by participating respiratory clinical teams, from those attending severe asthma services and undergoing nasal surgery as part of their comprehensive airways management, Additional patients will be identified by participating ENT teams, from those attending for surgery for nasal polyps, turbinectomy and septoplasties and reconstructive surgery.

Informed Consent Procedures and Screening:

On the day of scheduled surgery, potential participants interested in the study will be given the opportunity to ask questions from a GCP-trained member of the research team who may or may not be a member of the clinical team treating the participant. After questions have been answered potential participants will be asked to complete the written Informed Consent. Consenting participants will then be briefly screened to check they fulfil inclusion/exclusion criteria.

Data Collection (CRF):

A member of the site clinical team will complete the case report form (CRF) which contains only anonymised information but with a study code to link to patient samples (and to Consent Form for governance purposes). See Source Data section.

Sample Collection:

Excess nasal tissue will be resected by the ENT surgeons and placed in an appropriate container containing a buffered saline solution. Within two hours following the surgery, a member of the research team will collect the samples and transport them securely to the laboratory site adjacent to The Royal London Hospital (Blizard Institute, Barts and The London Medical School, Queen Mary University of London, 4 Newark Street, London E1 2AT).

Peripheral venous blood will be collected (optional) with standard aseptic technique. Nasal sections will be collected by nasoabsorption (optional) using nasal sponges placed into each nasal cavity for 5 minutes.

Subject Withdrawal:

In the event that a participant asks to withdraw from the study before the end of the Study Visit then the CRF and samples will be securely destroyed and no recording / analysis of data from that patient undertaken. In the event a participant asks to withdraw from the study after the first Study Visit has been completed the research team will be asked to withdraw any unreported linked data from that participant from the study.

End of Study Definition:

The end of the study is at completion of sample laboratory analysis for the last participant recruited. Statistical analysis of data may continue after the End of Study.

9. Statistical considerations

Associations and comparisons will be analysed with parametric and non-parametric statistical tests as appropriate. Diagnosed health conditions, presence/absence of infection, clinical markers of inflammation, and research laboratory immunological parameters will be analysed as variables likely to interact. Age and gender may also be considered.

As a laboratory research study with aim of revealing hypothesis-generating associations a formal power calculation is not possible. The sample size is based on previous experience with similar projects and feasibility.

10. Ethics

The Chief Investigator will ensure that the study is carried out in accordance with the ethical principles of the Research Governance Framework for Health and Social Care (Second Edition, 2005) and its subsequent amendments as applicable, as well as applicable legal and regulatory requirements. Study approval will include HRA and NHS REC approval, and they study will be conducted in accordance with GCP and declaration of Helsinki.

The research team has no financial or other competing interests for this study.

Human genome (gDNA) analysis will not be conducted as part of this research project.

Clinically defined immunodeficiencies will not be tested for as part of the immunological analyses.

The CRF and samples for immunological analysis will be anonymised so donors are not known to the research team or anyone reviewing the analyses and results.

Invitation to participate will be carried out in advance of scheduled surgery. Informed consent will be undertaken on the day of scheduled surgery. Potential participants will be given as long as they require to ask questions before deciding whether to participate. The study clinical procedure carries minimal risk. Donor decisions to participate or not in the study will not affect their health or clinical care. We therefore feel a short period for informed consent is appropriate for this low-risk study. Requiring potential volunteers to come back on a second occasion to give informed consent and samples would not facilitate ethical recruitment of patients, some of whom need to

travel significant distances for NHS appointments and some of whom have significant chronic ill health.

10.1. Annual Safety Reporting

The CI will send an Annual Progress Report to the REC and the sponsor using the HRA template on the anniversary of the REC “favourable opinion”.

11. Public involvement

No specific PPI has been done for this project due to its commercially translational nature, however, the objective of this study is well aligned to a key research priority of the European Asthma and Research Innovation Partnership (EARIP) to “Identify, understand and better classify the different forms of asthma, their progression, and effect on airway inflammation and the immune system”, these priorities were developed in close consultation with patient groups.

12. Data handling and record keeping

12.1. Data management

Clinical data will only be accessed by clinical site teams via the secure electronic medical records on NHS computers; and no identifiable information will be removed. All patient identifiable data will be anonymised and stored on secure NHS systems, which are password encrypted and only accessible by clinical staff.

Once data is anonymised, it will be moved using password-protected USB drives or secure nhs.net to nhs.net emails to university computers, based in the Blizard Institute, for data analysis. Data used on non-NHS systems, *e.g.* laboratory computers for data analysis, will be anonymised to protect confidentiality. Each participant will be assigned a study code that is separate from their identifiable data.

No personally identifiable information will leave the hospital. Consent forms will be stored in the study file in a locked cabinet at each study site. The only link between participant study codes and identifiable information will be a spreadsheet at site, which will be password-protected and held on secure NHS computers and in the secure site-file for NHS sites.

All NHS investigators undergo regular training in data confidentiality and information governance, in line with the trust policy on confidentiality. All investigators will be Good Clinical Practice or UK policy framework for health and social care research certified by the start date of the project.

All laboratory data generated by the study will be stored securely with a full audit trail.

12.2. Source Data

Source data (original data generated by study)

- Participant demographics (age and gender)
- Medical history of eczema, asthma, allergies, rhinitis
- Clinical reason for surgery
- Medication history (topical, nasal therapy or systemic corticosteroids; biologic therapy)
- Latest blood eosinophil count, FeNO (where available)

Source documents (data not generated by study)

- Patient medical records

12.3. Confidentiality

Information related to participants will be kept confidential and managed in accordance with the Data Protection Act, NHS Caldecott Principles, The Research Governance Framework for Health and Social Care, and the conditions of Research Ethics Committee Approval. Patients will be required to give their informed consent to allow site investigators to access their clinical records. Participants will be assigned a unique (alpha-numerical) identification code that will be used to label the sample. Only the study investigators will have access to the code. No identifiable participant data will be taken off secure NHS computers.

12.4. Record retention and archiving

When the research study is complete, study records will be kept for a further 5 years in accordance with the Research Governance Framework and Trust Policy. Records will be deposited in the Trust Modern Records Centre (Barts Health NHS Trust) and equivalent for other NHS sites. Consent forms will be stored in locked cabinets only accessible by the site investigators and researchers.

The lead investigator and lead researcher will have access to the samples and all anonymised research data for the duration of the study. All data generated will be anonymised and will be analysed at the Blizard Institute, Queen Mary's University of

London by the Lead Investigator and the research team associated with the study and collaborators. The research data will be archived for 5 years according to Queen Mary University of London/ Barts Health NHS Trust policy. The data will be archived in the Modern Records Facility, 9 Prescot Street, Aldgate, London, E1 8PR

13. Laboratories

13.1. Central and local laboratories

Sample processing and analysis will be performed in the Blizard Institute, 4 Newark Street, London E1 2AT. All research staff involved in the processing of the samples will have Good Laboratory Practice training.

13.2. Sample preparation and collection

Details of the sample collection (Sample ID and date of collection) will be recorded in a laboratory notebook as soon as samples arrive at the laboratory along with details of sample processing and storage. Patient identifiable information will not be made available to the research team.

13.3. Laboratory procedures

All laboratory procedures will be conducted purely for research purposes and not form part of any clinical care. Initial sample processing will be undertaken at the Blizard Laboratories, QMUL. Further pseudo-anonymised sample analyses by be undertaken by external collaborators where the assay cannot be conducted at QMUL.

Tissue samples will be dissociated by mechanical disruption. Immune cells may be isolated or enriched with magnetic beads and/or flow cytometry as per experimental protocols. Immune cells will be processed for single cell RNA sequencing using the 10X genomics platform at the genome centre core facility at the Blizard Institute. For bulk antibody repertoire analysis the tissue will be homogenized and RNA isolated from the homogenate. Antibody sequencing libraries will be generated by targeted PCR and then sequenced at the genome centre core facility at the Blizard Institute.

Subject to initial experiments being successful, B cell specificity will be interrogated using dCODE Klickmer oligonucleotide-barcoded antigens in later experiments, using antigens from viruses (e.g. covid, rhinovirus) and bacteria (e.g. Staphylococcus aureus enterotoxins [Chen et al. 2017]) known to trigger flares of nasal polyp inflammation and asthma exacerbations. dCODE oligonucleotide-barcoded putative auto-antigens,

identified as part of the ongoing GSK-funded IDEA ISS, will also be examined to compare tissue to blood B cell auto-antibody expression, and whether those B cells are IL-5 responsive.

Specific sequences will be analysed using an established computational pipeline based in the Immcantation packages (Nouri & Kleinstein. Bioinformatics 2018). Clonally-related B cell discerned by sequence identity (same VDJ gene rearrangement and CDRH3) and clonal lineages will be reconstructed. The properties of individual antibody repertoires will be analysed to quantify (per subclass) i) average clone sizes to provide a measure of clonal expansion, ii) clonal relatedness to other subclasses, and iii) frequencies of somatic hypermutation. These measures will then be compared between different patient groups (ex vivo WP) or stimulation condition (in vitro WP).

Samples may be sequenced *ex vivo* or after *in vitro* cell stimulations as described in the Study Design.

Other laboratory techniques that may be undertaken within the planned experimental work package:

- B cell flow-cytomtery
- ELISA for immunoglobulin proteins
- B-Spot cultures (B cell enzyme-linked immunosorbent spot (ELISpot))

Genomic DNA will not be analysed.

13.4. Sample storage and transfer

All samples will be processed within 24 hours of collection and processed sample material (e.g. excess RNA) stored at -80ºC in the laboratories within the Blizard Institute. Samples will be stored in sample preservation buffer overnight prior to processing. The details of the stored samples will be recorded in an electronic database in an anonymised form.

14. Safety reporting

The only patient contact in this study is the consent process and the sample donation of residual tissue collected as part of clinical care. As such study-attributable Adverse Events (AE) and Serious Adverse Events (SAE) are extremely unlikely. In the event of an AE or SAE then this will reported to the CI by any research team members, and escalated as appropriate by the CI. Unsolicited GSK product related events will be reported according to local reporting regulations.

Notification and Reporting of Serious Adverse Events:

Serious Adverse Event (SAEs) that are considered to be 'related' and 'unexpected' will be reported to the sponsor within 24 hours of learning of the event and to the Main REC within 15 days in line with the required timeframe.

In addition to reporting to the sponsor, any SAEs (anonymised information) will be reported to the funder.

15. Monitoring and auditing

The Sponsor or delegate retains the right to audit any study, study site or central facility. In addition, any part of the study may be audited by the funders where applicable.

The study will be monitored according to our sponsor's SOP (QMUL).

16. Study committees

All data generated as part of this study will undergo regular review by an informal committee within the research department of the Chief Investigator, including external collaborators. This committee will comprise the Chief Investigator and researcher(s) involved in the study as well as independent senior members of the Department who will review and discuss data and research outcomes.

17. Finance and funding

This study is funded by a research grant from GSK.

18. Insurance and indemnity

The insurance that Queen Mary has in place provides cover for the design and management of the study as well as "No Fault Compensation" for participants, which provides an indemnity to participants for negligent and non-negligent harm.

19. Dissemination of research findings

Research findings will be disseminated by publication in peer-reviewed journals and presentation at local, national and international research meetings and conferences.

20. References

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