

## New biologic (Ab-IPL-IL-17) for IL-17-mediated diseases

Saviano, Anella; Manosour, Adel Abo; Raucci, Federica; Merlino, Francesco; Marigliano, Noemi; Schettino, Anna; Wahid, Mussarat; Begum, Jenefa; Filer, Andrew; Manning, Julia E; Casillo, Gian Marco; Piccolo, Marialuisa; Ferraro, Maria Grazia; Marzano, Simona; Russomanno, Pasquale; Bellavita, Rosa; Irace, Carlo; Amato, Jussara; Alfaifi, Mohammed; Rimmer, Peter

DOI:  
[10.1136/ard-2023-224479](https://doi.org/10.1136/ard-2023-224479)

License:  
Creative Commons: Attribution-NonCommercial (CC BY-NC)

*Document Version*  
Peer reviewed version

*Citation for published version (Harvard):*  
Saviano, A, Manosour, AA, Raucci, F, Merlino, F, Marigliano, N, Schettino, A, Wahid, M, Begum, J, Filer, A, Manning, JE, Casillo, GM, Piccolo, M, Ferraro, MG, Marzano, S, Russomanno, P, Bellavita, R, Irace, C, Amato, J, Alfaifi, M, Rimmer, P, Iqbal, T, Pieretti, S, Vellecco, V, Caso, F, Costa, L, Giacomelli, R, Scarpa, R, Cirino, G, Bucci, M, McGettrick, HM, Grieco, P, Iqbal, AJ & Maione, F 2023, 'New biologic (Ab-IPL-IL-17) for IL-17-mediated diseases: identification of the bioactive sequence (nIL-17) for IL-17A/F function', *Annals of the Rheumatic Diseases*. <https://doi.org/10.1136/ard-2023-224479>

[Link to publication on Research at Birmingham portal](#)

### General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

## **New biologic (Ab-IPL-IL17™) for IL-17-mediated diseases: Identification of the bioactive sequence (nIL-17™) for IL-17A/F function.**

Anella Saviano<sup>1,†</sup>, Adel Abo Manosour<sup>2,†</sup>, Federica Raucci<sup>1,†</sup>, Francesco Merlino<sup>3</sup>, Noemi Marigliano<sup>1</sup>, Anna Schettino<sup>1</sup>, Mussarat Wahid<sup>4</sup>, Jeneba Begum<sup>5</sup>, Andrew Filer<sup>4</sup>, Julia E Manning<sup>4</sup>, Gian Marco Casillo<sup>3</sup>, Marialuisa Piccolo<sup>6</sup>, Maria Grazia Ferraro<sup>6</sup>, Simona Marzano<sup>3</sup>, Pasquale Russomanno<sup>3</sup>, Rosa Bellavita<sup>3</sup>, Carlo Irace<sup>6</sup>, Jussara Amato<sup>3</sup>, Mohammed Alfaifi<sup>2</sup>, Peter Rimmer<sup>5,7</sup>, Tariq H Iqbal<sup>7,8</sup>, Stefano Pieretti<sup>9</sup>, Valentina Vellecco<sup>3</sup>, Francesco Caso<sup>10</sup>, Luisa Costa<sup>10</sup>, Roberto Giacomelli<sup>11,12</sup>, Raffaele Scarpa<sup>10</sup>, Giuseppe Cirino<sup>3</sup>, Mariarosaria Bucci<sup>3</sup>, Helen M McGettrick<sup>4,‡</sup>, Paolo Grieco<sup>3,‡</sup>, Asif Jilani Iqbal<sup>5,1,‡,\*</sup> and Francesco Maione<sup>1,‡,\*</sup>.

<sup>1</sup>*ImmunoPharmaLab*, Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Via Domenico Montesano 49, 80131, Naples, Italy.

<sup>2</sup>Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid University, Abha, Saudi Arabia.

<sup>3</sup>Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Via Domenico Montesano 49, 80131, Naples, Italy.

<sup>4</sup>Institute of Inflammation and Ageing (IIA), College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2WB, UK.

<sup>5</sup>Institute of Cardiovascular Sciences (ICVS), College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2TT, UK.

<sup>6</sup>*BioChemLab*, Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Via Domenico Montesano 49, 80131, Naples, Italy.

<sup>7</sup>Department of Gastroenterology, Queen Elizabeth Hospital Birmingham NHS Foundation Trust, Birmingham, UK.

<sup>8</sup>Institute of Microbiology and Infection (IMI), College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2TT, UK.

<sup>9</sup>National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy.

<sup>10</sup>Rheumatology Research Unit, Department of Clinical Medicine and Surgery, University of Naples Federico II, via S. Pansini 5, 80131 Naples, Italy.

<sup>11</sup>Clinical and Research Section of Rheumatology and Clinical Immunology, Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Italy.

<sup>12</sup>Rheumatology and Clinical Immunology, Department of Medicine, University of Rome "Campus Bio-Medico", School of Medicine, Rome, Italy.

<sup>†</sup>These authors share first authorship.

†These authors share senior authorship.

**\*Author for correspondence:** **Francesco Maione**, Head of *ImmunoPharmaLab*, Department of Pharmacy, School of Medicine, University of Naples Federico II, Via Domenico Montesano 49, 80131, Naples, Italy. E-mail: [francesco.maione@unina.it](mailto:francesco.maione@unina.it). **Asif Jilani Iqbal**, Institute of Cardiovascular Sciences (ICVS), College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2TT, UK. E-mail: [a.j.iqbal@bham.ac.uk](mailto:a.j.iqbal@bham.ac.uk).

**Author Contributions:** This was a community-based project that was only possible through unique combinations of expertise from multiple groups. As a result, there were many equal contributions, and we would like to acknowledge that no single ordering of authors could have captured the importance of each contribution. A.Sa., A.A.M., F.R., F.Me. N.M, M.W. and H.M.M. performed the *in vivo* and *ex vivo* experiments and data analysis. A.Sc., M.W., J.B., A.F., J.E.M., G.M.C., M.P., M.G.F., S.M., P.Ru., R.B., C.I., J.A., F.C., R.G., R.S., M.A., P.Ri., T.H.I., S.P. and V.V. provided technical help and performed *in vitro* and *ex vivo* experiments. F.C., L.C., G.C., M.B., P.G., H.M.M., A.J.I. and F.Ma. provided expertise and drafted the manuscript. P.G., H.M.M., A.J.I. and F.Ma. revised the final version of the manuscript for intellectual contents. All authors discussed the results and commented on the article. All authors gave final approval to the publication.

**Competing Interest Statement:** This article has been conducted and written in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors A.Sa., F.R., F.Me., R.B., P.G., M.B., A.J.I. and F.Ma. hold patents for the diagnostic and therapeutic use of nIL-17™ and Ab-IPL-IL17™ (IT patent No. 102022000016722) in autoimmune disease, chronic inflammatory disease and other diseases in which IL-17 producing cells contribute to pathogenesis. All other authors have no conflicts of interest.

**Keywords:** Autoimmune diseases; Inflammation; IL-17; leukocytes; fibroblast.

**Key messages:** Assessment of the therapeutic efficacy of Ab-IPL-IL17™ in immune-mediated inflammatory diseases.

- **What is already known on this topic** – Interleukin (IL)-17s cytokine (IL-17A, IL-17F and heterodimer IL-17A/F) are key drivers of inflammation that are functionally dysregulated in several human immune-mediated inflammatory diseases (IMIDs), such as rheumatoid arthritis (RA), psoriasis, and inflammatory bowel disease (IBD).
- **What this study adds** – In this study, we identified for the first time the “essential” amino acid sequence (nIL-17™) responsible for IL-17A/F biological activity in both mouse and human. We have taken advantage of this knowledge to generate a novel antibody (Ab-IPL-IL17™) that specifically targets the active nIL-17™ peptide sequence and has utility for understanding IL-17A/F biology/pathogenesis in mouse/human.
- **How this study might affect research, practice, or policy** – We demonstrate that Ab-IPL-IL17™ is as effective as reference anti-IL17 antibodies in reducing inflammatory processes, in preclinical models of IMIDs and in human clinical samples from inflammatory bowel disease (IBD) and rheumatoid arthritis (RA). Importantly, Ab-IPL-IL-17™ exhibited, in mice, significantly more neutralising activity limiting inflammation and disease progression, with lower immunogenicity and adverse haematological side effects when compared to reference antibodies.

## ABSTRACT

*Objectives.* Interleukin (IL)-17s cytokine are key drivers of inflammation that are functionally dysregulated in several human immune-mediated inflammatory diseases (IMIDs), such as rheumatoid arthritis (RA), psoriasis, and inflammatory bowel disease (IBD). Targeting these cytokines has some therapeutic benefit, but issues associated with low therapeutic efficacy and immunogenicity for subgroups of patients or IMIDs reduces their clinical use. Therefore, there is an urgent need to improve the coverage and efficacy of antibodies targeting IL-17A and/or IL-17F and IL-17A/F. *Methods and Results.* Here, we initially identified a bioactive 20 amino acid IL-17A/F-derived peptide (nIL-17™) that mimics the pro-inflammatory actions of the full-length proteins. Subsequently, we generated a novel anti-IL-17 neutralising monoclonal antibody (Ab-IPL-IL17™) capable of effectively reversing the pro-inflammatory, pro-migratory actions of both nIL-17™ and IL-17A/F. Importantly, we demonstrated that Ab-IPL-IL17™ has less off-target effects than the current gold-standard biologic, secukinumab. Finally, we compared the therapeutic efficacy of Ab-IPL-IL-17™ with reference anti-IL-17 antibodies in preclinical murine models and samples from RA and IBD patients. We found that Ab-IPL-IL17™ could effectively reduce clinical signs of arthritis and neutralise elevated IL-17 levels in IBD patient serum. *Conclusions.* Collectively, our preclinical and *in vitro* clinical evidence indicates high efficacy and therapeutic potency of Ab-IPL-IL17™, supporting the rationale for large-scale clinical evaluation of Ab-IPL-IL17™ in patients with IMIDs.

## MAIN TEXT

### INTRODUCTION

Evidence from basic research and clinical trials demonstrates that the interleukin (IL)-17 immune axis exerts distinct biological effects dependent upon the tissue or disease context (1). IL-17-producing T cells (Th17) and innate immune cells (including neutrophils, monocytes, and macrophages) play key protective roles in the immune response to various microbial pathogens (2). However, IL-17-driven responses are responsible for tissue damage linked to infection-associated immunopathology and can result in the development of immune-mediated inflammatory diseases (IMIDs) (3, 4), such as psoriasis, psoriatic arthritis (PsA), rheumatoid arthritis (RA), inflammatory bowel disease (IBD) and ankylosing spondylitis (AS) (5). Dysregulation of IL-17A (and in a less extent, IL-17F and IL-17A/F) production and/or binding to its receptor(s) have been associated with IMID pathology (6), making this complex an attractive target for therapeutic interventions (7). Indeed, secukinumab, ixekizumab (anti-IL-17A antibodies) and bimekizumab (anti-IL-17A/F antibody) are already effective in treating plaque psoriasis, PsA and AS (7). Despite the potent blockade of cytokine signalling offered by these biological therapies, many patients have only partial or transient responses associated with various side effects. Therefore, identifying potential novel therapeutic targets or optimising those already available is urgently needed and will likely have a significant clinical benefit (5, 8).

IL-17 is composed of six family members, of which IL-17A and IL-17F are predominantly involved in driving inflammatory responses (4). Convincing evidence indicates that IL-17A/F use their C-terminal sequence to bind the heterodimeric receptor IL-17RA and IL-17RC (9). Upon binding, this receptor complex recruits the ubiquitin ligase Act-1 (via the SEF/IL-17R domain) (10), which in turn recruits tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) receptor-associated factor 6 (TRAF6), leading to the activation of nuclear factor kappa B (NF $\kappa$ B) and the mitogen-activated protein (MAP) kinase pathways. Activation of these pathways generates a plethora of inflammatory mediators, such as IL-1 $\alpha/\beta$ , IL-6, IL-8 and TNF- $\alpha$  (1, 11, 12), which contribute to pathological processes in various IMIDs. Identification of the key active amino acids in the C-terminal sequence of IL-17A/F could, therefore, prove critical in generating a more biologically active neutralising antibody with reduced off-target effects (13).

Exploring murine and human IL-17A/F protein sequences, we have identified an essential 20-mer IL-17-derived peptide (nIL-17<sup>TM</sup>) that is responsible for the bioactivity of IL-17A, IL-17F and/or IL-17A/F heterodimer, mimicking a range of actions elicited by the full-length cytokines. Specifically, we demonstrate that nIL-17<sup>TM</sup> activates IL-17RA/C-dependent intracellular signalling to induce activation of NIH-3T3 mouse embryonic fibroblast cells and human dermal blood endothelial cells (HDBEC) leading to increased cytokine, chemokine, and adhesion molecule expression. Additionally, nIL-17<sup>TM</sup> promoted leukocyte recruitment to pre-inflamed tissues *in vivo* (air pouch model) and *in vitro* (to inflamed endothelium). Subsequently, we developed a monoclonal neutralising antibody (Ab-IPL-IL-17<sup>TM</sup>) targeting nIL-17<sup>TM</sup>, which effectively reversed the actions of nIL-17<sup>TM</sup> leading to reductions in chemokine, cytokine, and adhesion molecule levels on target cells, as well as reducing the inflammation infiltrate. Finally, we compared the therapeutic efficacy of Ab-IPL-IL-17<sup>TM</sup> with reference anti-IL-17 antibodies in preclinical models of IMIDs, specifically arthritis and inflammatory bowel disease (IBD). Crucially, Ab-IPL-IL-17<sup>TM</sup> exhibited significantly more neutralising activity limiting inflammation and disease progression, with lower immunogenicity and adverse haematological side effects when compared to reference antibodies. Future studies and clinical trials will need to address the varying requirements of Ab-IPL-IL-17<sup>TM</sup> as an alternative biological therapy for treating patients with IMIDs.

## RESULTS

### Characterising the bioactive sequence in IL-17A and IL-17F

To identify the bioactive portion within IL-17A and IL-17F, we designed a series of peptides (**Fig. S1A-C**), of different lengths, which mimic the C-terminal region of IL-17A/F, considered as essential in the interaction with the receptor counterpart. These truncated peptides would meet the affinity/receptor interaction requirements of both murine and human IL-17A/F for their cognate receptors (3, 4). Subsequently, we assessed the ability of these peptides to mimic the actions of native IL-17A, IL-17F and IL-17A/F heterodimer to induce IL-6 production from a murine embryonic fibroblast cell line NIH-3T3 (14). Fibroblasts are a major cellular target for IL-17A, leading to the generation of several inflammatory cytokines (e.g., IL-6 and IL-8) which drive the local inflammatory response (9). We found that only peptide 2 (named nIL-17<sup>TM</sup>) was able to promote IL-6 release to a greater extent when compared to both IL-17A and, IL-17F at similar molar concentrations (from 0.610 to 0.725 nM) (**Fig. 1A, Fig. S1D**). nIL-17A<sup>TM</sup> displayed similar biological activity to the recombinant full-length native IL-17A/F heterodimer (**Fig. 1A**).

Furthermore, our *in vitro* cytotoxic examination revealed a safe profile for nIL-17<sup>TM</sup> in tested concentration on NIH-3T3 cell lines (**Fig. S2**). Moreover, modifications through the replacement of carboxy (-CO<sub>2</sub>H) with amide (-CONH<sub>2</sub>) group at C-terminal (nIL-17A<sup>TM</sup>-NH<sub>2</sub>), removal of any tertiary structure by denaturing the peptide (nIL-17A<sup>TM</sup>-DN) or scrambling the amino acid sequence (nIL-17A<sup>TM</sup>-SC) had no effect on IL-6 production by NIH-3T3 (**Fig. 1B**). These data demonstrate for the first time that a 20-mer sequence from both murine and human IL-17A/F is responsible for IL-17s biological activity.

IL-17A, and to a lesser extent IL-17F and the heterodimer IL-17A/F, binding to the IL-17 receptor complex (IL-17RA/RC) leads to the recruitment of Act-1/TRAF6 and, ultimately, activation of inflammatory transcription factors via NFκB to induce gene expression (15). Indeed, we found that nIL-17<sup>TM</sup> further amplified the expression of both Act-1 and NFκB, but not IL-17RA or IL-17RC, when compared to full-length native IL-17A protein (**Fig. 1C-D; Fig. S3**). A similar observation was reported in mouse embryonic fibroblasts (NIH-3T3) and mouse macrophages (J774A.1) where both IL-17RA and IL-17RC expression remained unchanged following treatment with IL-17A (14, 16). To confirm peptide-receptor interactions, biotinylated native IL-17A and IL-17F protein or nIL-17A<sup>TM</sup> were incubated with either mouse or human IL-17RA or IL-17RC and binding was assessed. nIL-17A<sup>TM</sup> displayed similar binding profiles to both receptors as was seen with full-length IL-17A/F (**Fig. 1E, Fig. S4**, respectively).

To gain a better understanding of how nIL-17<sup>TM</sup> binds IL-17RA and IL-17RC receptors at an atomic level, molecular docking studies were performed. The 3D structure of nIL-17<sup>TM</sup> peptide was predicted using the PEP-FOLD 4 computational tool (17) and experimentally analysed by circular dichroism (CD) (**Fig. S5-I-A**). Using the BeStSel method (18), we selected a structural model of nIL-17<sup>TM</sup> that consisted of two β-strands followed by a short α-helix (**Fig. S5-I-B; Auxiliary Table 1**). This model showed the closest structural similarity to the receptor-binding region of human IL-17A. The 3D structures of IL-17RA and IL-17RC proteins were obtained from the crystal structure of the two receptors in complex with human IL-17A (Protein Data Bank id: 7ZAN), also considering the similarity between IL-17RC isoform 1 used in our biological tests and isoform 2 present in the 3D structure (**Auxiliary Table 2**). Subsequent docking analysis predicted that nIL-17<sup>TM</sup> interacts with IL-17RA and IL-17RC in a manner similar to that of the C-terminal region of IL-17A, whose sequence it mimics (**Fig. S5-II**). In particular nIL-17<sup>TM</sup> interacts with the IL-17RA binding pocket between two type III fibronectin domains, with D1 binding the N-terminal region of nIL-17<sup>TM</sup> and D2 interacting mainly with the C-terminal α-helix (**Fig. S5-III**). Whilst the predicted binding of nIL-17<sup>TM</sup> was mostly superimposable with IL-17A homodimer, there were

significant differences in the positioning of nIL-17<sup>TM</sup> N-terminal region and in the interaction with the receptor amino acids (**Fig. S5-III-B-C**). The predicted binding mode of nIL-17<sup>TM</sup> with IL-17RC was similar to that seen for IL-17RA, except no interactions between the N-terminal region of nIL-17<sup>TM</sup> and the D1 domain were predicted (**Fig. S5-IV**). The higher affinity of nIL-17<sup>TM</sup> for IL-17RA compared to the other peptides and the IL-17A C-terminal region may be attributable to the multiple interactions formed with the D2 domain that anchors the peptide to the receptor. This binding mode may be possible due to the peculiar structural conformation of nIL-17<sup>TM</sup>, permitting closure of the binding site between the two type III fibronectin domains (**Auxiliary Fig. 1**), thereby improving the biological activity of nIL-17<sup>TM</sup> compared to other peptides (**Fig. S1**). Conversely, the limited interaction between nIL-17<sup>TM</sup> and IL-17RC, may in part be due to the lack of closure of the binding cavity (**Auxiliary Fig. 2**), which may explain the differential binding activity of nIL-17<sup>TM</sup> towards the two receptors. Collectively, these data support our hypothesis that the bioactive region within both IL-17A and IL-17F meets the affinity/receptor interaction requirements of both murine and human cognate receptors.

### **nIL-17<sup>TM</sup> is a potent activator of inflammatory response**

IL-17A, and in part IL-17F, can modulate a variety of leukocyte functions, having a broad and wide-ranging impact on inflammatory responses (19). In the context of the myeloid lineage, it has previously been shown that treatment with IL-17A can amplify the production of inflammatory cytokines from human M1 macrophages (20). In agreement with this study, nIL-17<sup>TM</sup> significantly increased IL-6 and TNF- $\alpha$  release from M1 macrophages to a similar degree as seen with native full-length IL-17A (**Fig. 1F-G**). In line with previous studies (21), this response was specific for M1 macrophages, and not seen in M0 or M2 macrophages (**Fig. S6A-B**). Furthermore, there were no intrinsic differences in IL-17RA or IL-17RC expression following IL-17A or nIL-17<sup>TM</sup> treatment on any of the macrophage subsets (**Fig. S6C-E**). These data demonstrate that nIL-17<sup>TM</sup> retains a similar inflammatory amplification activity as that observed with the native full-length protein.

In the context of inflammatory cell recruitment, IL-17A, but not IL-17F, can directly function as a chemotactic agent for neutrophils promoting their entry into inflamed tissues (22). Moreover, IL-17A acts synergistically with TNF- $\alpha$  to increase the expression of neutrophil capture receptors (E-, P-selectin) and presentation of neutrophil specific chemokines (CXCL [chemokine C-X-C motif ligand] 1, 2, 8) by HDBEC (23). We, therefore, assessed the chemotactic potential of nIL-17<sup>TM</sup> to drive neutrophil migration through inflamed endothelial cells (**Fig. 1H**). Notably, we found that nIL-17<sup>TM</sup> significantly increased neutrophil migration in a dose-dependent manner, unlike native IL-17A (**Fig. 1H**). Interestingly, 500 ng/ml nIL-17<sup>TM</sup> had a greater chemotactic capacity to drive neutrophil migration when compared to native IL-17A at the same concentration (**Fig. 1H**).

To validate these findings *in vivo*, we utilised the myeloid-driven sub-chronic model of inflammation, the mouse dorsal air pouch (**Fig. 2A**), where we previously demonstrated that IL-17A preferentially increases the recruitment of pro-inflammatory Ly6C<sup>hi</sup> monocytes and Gr1<sup>+</sup> neutrophils in acute and chronic inflammatory settings (16, 24, 25). Native murine IL-17A or nIL-17<sup>TM</sup> were administered on day 6 following establishment of the air pouch. We observed a significant increase in CD45<sup>+</sup> leukocytes recruited in response to IL-17A, which was further exacerbated (~48%) in the presence of nIL-17<sup>TM</sup> (**Fig. 2B**). Moreover, in the presence of a commercially available neutralising IL-17A monoclonal antibody (MAB421) this effect was lost (**Fig. S7**). Such effects have been previously described in models of endotoxin-induced lung inflammation (26). Interestingly, blocking the neutrophil (IL-8/KC [keratinocyte-derived cytokine]) or monocyte (JE/MCP-1 [monocyte chemoattractant protein-1]) chemokines simultaneously with administering nIL-17<sup>TM</sup> significantly impaired leukocyte recruitment to the air pouch (**Fig. S7**), indicating that nIL-17<sup>TM</sup> drives neutrophil and monocyte infiltration via indirect release of these chemoattractants (16). Indeed, previous reports have shown that IL-17A driven neutrophil transmigration through TNF- $\alpha$ -stimulated murine endothelial cells or resting human lung

microvascular endothelial cells *in vitro* was completely abolished when chemokine receptor (CXCR)<sup>2-/-</sup> neutrophils were perfused over the cultures (23) or when cultures were treated with neutralising antibodies against CXCL8/IL-8 (27). We further corroborated this idea through proteome analysis of cyto-chemokines released locally in response to either IL-17A or nIL-17<sup>TM</sup> (Fig. 2C-F). Importantly, nIL-17<sup>TM</sup> augments the amount of several pro-inflammatory mediators, such as IL-8/KC, MCP-1/JE, soluble intercellular adhesion molecule 1 (sICAM1) (28), when compared to native IL-17A (Fig. 2F). Others have reported similar IL-17-induced increases in inflammatory mediators (KC, JE, IFN- $\gamma$ , IL-1 $\alpha$ , sICAM1, MIPs [macrophage inflammatory proteins], IL-6) within a variety of tissues, including pouch cavities, brain, blood, and aorta (16, 29, 30). Taken together, these data reinforce our earlier observations that nIL-17<sup>TM</sup> is a more potent pro-inflammatory stimulus than native full-length IL-17A protein and confirm that this peptide truly represents the most biological active sequence of this cytokine.

### **nIL-17<sup>TM</sup> amplifies endothelial cell activation to support leukocyte trafficking**

IL-17 receptors are expressed by both hematopoietic cells of the immune system and by stromal cells, such as HDBEC (27, 31). IL-17A synergistically amplifies HDBEC response to TNF- $\alpha$ , further increasing expression of the adhesion molecules ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) (23, 27). Given this, the effects we describe above for nIL-17<sup>TM</sup> could be due to a direct effect on the leukocytes or the HDBEC alone or on both cell types. To address this, we analysed the adhesion (phase bright) and transmigration (phase dark) of peripheral blood mononuclear cells (PBMC) across inflamed endothelial cells (ECs) following the addition of IL-17A or nIL-17<sup>TM</sup> (Fig. 2G). As we have previously published (32), TNF- $\alpha$  stimulation enhanced PBMC adhesion, but this was not further amplified in the presence of either IL-17A protein or nIL-17<sup>TM</sup> peptide (Fig. 2H). In agreement with our findings, IL-17 did not enhance absolute numbers of adherent leukocytes (neutrophils) to TNF- $\alpha$  stimulated endothelium *in vitro* or *in vivo*, but rather altered cellular behaviour, increasing the number undergoing transmigration (23). Like earlier findings, nIL-17<sup>TM</sup> exacerbated PBMC migration through inflamed endothelium to the same extent as seen with full-length IL-17A (Fig. 2I). PBMC capture and firm adhesion are mediated through E-selectin and VCAM-1 expressed by the inflamed endothelium (32), with VCAM-1 levels remaining unaffected by the addition of either IL-17A protein or peptide in combination with TNF- $\alpha$  when compared to TNF- $\alpha$  alone (Fig. 2J) in agreement with previous publications (23). Of note, neither IL-17A protein or peptide induced VCAM-1 expression in the absence of TNF- $\alpha$ . By contrast, PBMC transmigration is dependent on  $\beta_2$ -integrins binding endothelial ICAM-1 (33), with the protein for the latter being synergistically elevated by both IL-17A or nIL-17<sup>TM</sup> (Fig. 2K). No differences were observed in IL-17RA or RC expression on endothelial cells under any conditions used (Fig. S8A-B). These findings demonstrate that nIL-17<sup>TM</sup> enhances endothelium activation in response to inflammation, to further amplify leukocyte migration.

### **nIL-17<sup>TM</sup> specific antibody (Ab-IPL-IL-17<sup>TM</sup>) displays potent neutralising activity**

Given that nIL-17<sup>TM</sup> clearly demonstrates a more prominent inflammatory activity than full-length IL-17A, we embarked on generating a novel IL-17 neutralising antibody: Ab-IPL-IL-17<sup>TM</sup> targeting the nIL-17<sup>TM</sup> sequence (IT patent No. 102022000016722). Ab-IPL-IL-17<sup>TM</sup> significantly decreased IL-6 production from (commercially available) IL-17A homodimer and nIL-17<sup>TM</sup>-stimulated NIH-3T3 cells in a concentration-dependent manner (Fig. 3A) without any cytotoxic effect for all tested concentrations on murine embryonic fibroblast cell lines (Fig. S9); reduced binding affinity of IL-17A to IL-17RA or RC (Fig. 3B); blocked the production of IL-6 and TNF- $\alpha$  from IL-17A-treated M1 macrophages (Fig. 3C-D respectively); and decreased IL-17A-induced neutrophil migration (Fig. 3E). *In vivo*, Ab-IPL-IL-17<sup>TM</sup> simultaneously administered with IL-17A reduced influx of total CD45<sup>+</sup> leukocytes into the inflamed air pouch (Fig. 3F), with a corresponding reduction observed in several cyto-chemokines (Fig. 3G). Similar observations were made *in vitro*, where pre-treatment with Ab-IPL-IL-17<sup>TM</sup> significantly reduced both PBMC



and PBL adhesion to and transmigration through IL-17+TNF- $\alpha$  treated endothelium (**Fig. 3H-J** and **Fig. S10**, respectively), which was mirrored by decreased HDBEC expression of VCAM-1 and ICAM-1 (**Fig. 3K-L**).

#### **Ab-IPL-IL-17<sup>TM</sup> maintains activity in the absence of off-target immunogenic effects as seen with secukinumab**

Current anti-IL-17A therapies (secukinumab and ixekizumab) are associated with unwanted off-target immunogenic effects, lymphocytosis, and thrombocytopenia (34). As such, numerous clinical trials are currently investigating new biologic therapies targeting IL-17 biology/function to improve clinical outcomes in patients with IMIDs (35). Here, we evaluated *in vivo* the neutralising potential of Ab-IPL-IL-17<sup>TM</sup> on IL-17A, IL-17F and IL-17A/F production. Ab-IPL-IL-17<sup>TM</sup> was able to significantly reduce the plasma concentration of IL-17A and IL-17F to similar levels as seen with secukinumab (**Fig. 4A-B**). Notably, Ab-IPL-IL-17<sup>TM</sup> significantly reduced the plasma concentration of IL-17A/F heterodimer to a similar extent as the reference anti-IL-17A/F antibody bimekizumab (**Fig. 4C**). To assess immunogenicity under homeostatic conditions, we administered a single dose of Ab-IPL-IL-17<sup>TM</sup> and measured total IgG and IgG1 levels over 21 days, comparing levels with the reference anti-IL-17 antibody (MAB421) and the current gold standard clinical therapies secukinumab and bimekizumab (**Fig. 4D-E**). A significant increase in total IgG and IgG1 was observed at 72 h and remained elevated until day 14 with secukinumab, but no increase was observed in mice injected with Ab-IPL-IL-17<sup>TM</sup> (**Fig. 4D-E**) or bimekizumab treatment. Whole blood analysis revealed that secukinumab and MAB421 increased total circulating lymphocyte numbers 72 h post-injection/administration, which remained significantly elevated up to 7 days when compared to vehicle control (**Fig. 4F**). Furthermore, secukinumab induced thrombocytopenia as early as 24 h and platelet numbers remained significantly reduced at 72 h and 7 days post administration (**Fig. 4G**). Strikingly, Ab-IPL-IL-17<sup>TM</sup> and the selective IL-17A/F neutralizing antibody bimekizumab had no effect on total lymphocyte or platelet numbers at any time point assessed (**Fig. 4F-G**). No changes were observed for other hematological parameters (**Table 1**). Collectively these data show that Ab-IPL-IL-17<sup>TM</sup> retains strong neutralising activity without triggering unwanted immunogenic response, making it an attractive clinical therapy.

#### **Ab-IPL-IL-17<sup>TM</sup> reduces pathological symptoms of arthritis and inflammatory bowel disease**

Secukinumab and ixekizumab are current therapies for Ps and AS, therefore, we investigated the clinical efficacy of Ab-IPL-IL-17<sup>TM</sup> in preclinical murine models of arthritis and *ex vivo* analysis of blood or cells isolated from tissues of patients with RA or IBD. Excitingly we found that therapeutic administration of Ab-IPL-IL-17<sup>TM</sup> significantly reduced joint swelling in the murine antigen-induced arthritis (AIA) model (**Fig. 5A-B**). Indeed, treating AIA with commercially available neutralising antibodies to IL-17 has been previously reported to reduce clinical symptoms of arthritis (36) and neutrophil accumulation within the joint (37). It is important to note that Ab-IPL-IL-17<sup>TM</sup> was as effective at halting disease progression and triggering resolution as the gold-standard current treatment for RA, infliximab (**Fig. 5A-B**) with a significant reduction in infiltrating neutrophils (**Fig. 5C**). and monocytes (**Fig. 5D**) observed.

As a proof-of-concept of our investigation, we next analysed the potential clinical benefit of Ab-IPL-IL-17<sup>TM</sup> in the treatment of patients with RA and IBD using *ex vivo* analysis of patient materials ensuring an equal proportion of male and female donors. Firstly, we tested the actions of Ab-IPL-IL-17<sup>TM</sup> on fibroblasts obtained from treatment naïve patients with acutely resolving arthritis or persistent RA. These isolated cells constitutively release IL-6 and IL-8 when in culture, and this was significantly increased when the fibroblasts were stimulated with recombinant IL-17 and TNF- $\alpha$  (**Fig. 5E-F**). Others have shown IL-17-induced increases in IL-6 and IL-8 gene

expression and secretion from fibroblasts isolated from treated RA patients undergoing joint replacement surgery (38). In agreement with our data, a combination treatment of IL-17 with TNF- $\alpha$  amplified IL-8 gene expression, but in contrast with our findings no changes in IL-6 were observed. Ab-IPL-IL-17<sup>TM</sup> and the reference function blocking anti-IL-17 antibody (MAB317) were able to reverse this effect in inflamed RA fibroblasts to a similar degree, such that significantly lower concentrations of IL-6 and IL-8 were released (Fig. 5E-F). By contrast, neither MAB317 nor Ab-IPL-IL-17<sup>TM</sup> altered the IL-6 or IL-8 secretion from IL-17 + TNF- $\alpha$  activated resolving fibroblasts (Fig. 5E-F). These data strongly indicate the Ab-IPL-IL-17<sup>TM</sup> therapy specifically inhibits the pro-inflammatory actions of chronically inflamed fibroblasts within the rheumatoid joint but does not adversely alter the protective response elicited during acutely resolving joint inflammation. In the context of IBD, treatment with several IL-17 neutralising antibodies has been shown to have limited efficacy with some patients completely refractory to treatment (39). In a proof-of-concept experiment, Ab-IPL-IL-17<sup>TM</sup> was able to deplete/sequester plasma IL-17A within samples obtained from treatment naïve IBD patients attending an inception clinic (Fig. 5G) but had no effect on plasma IL-6 or TNF- $\alpha$  concentrations (Fig. S11). These data indicate that Ab-IPL-IL-17<sup>TM</sup> has the potential to effectively alleviate pathological pro-inflammatory responses in patients with IMIDs.

## DISCUSSION

Whilst current biologics targeting IL-17A/F exist for the treatment of various IMIDs, issues with immunogenicity, partial/incomplete patient responses and adverse side effects are driving the field to design and develop more effective biologics (5). Here, we initially identified a bioactive 20-mer IL-17A/F-derived peptide (nIL-17<sup>TM</sup>) that mimics the pro-inflammatory actions of the full-length proteins. Subsequently, we generated a novel anti-IL-17 neutralising monoclonal antibody (Ab-IPL-IL17<sup>TM</sup>) capable of effectively reversing the pro-inflammatory, pro-migratory actions of nIL-17<sup>TM</sup>. Importantly, we demonstrated that in mice Ab-IPL-IL17<sup>TM</sup> has less hematological off-target effects than the current gold-standard biologic, secukinumab. Finally, we found that Ab-IPL-IL17<sup>TM</sup> effectively reduced clinical signs of experimental arthritis, decreased *in vitro* pro-inflammatory cytokine production by synovial fibroblast cells and neutralised elevated IL-17 levels in IBD patient serum following ex-vivo stimulation. Collectively, our preclinical and *in vitro* clinical evidence indicates high efficacy and therapeutic potency of Ab-IPL-IL17<sup>TM</sup>, supporting the rationale for large-scale clinical evaluation of Ab-IPL-IL17<sup>TM</sup> in patients with IMIDs.

Currently numerous therapeutics targeting IL-17/IL-17R pathway for the treatment of IMIDs are available, including several approved monoclonal antibodies (secukinumab and ixekizumab) and several newer biologics under clinical trials, such as bimekizumab (8, 13, 40). However, some of these have been discontinued in certain patient groups due to severe/damaging side effects: increased intestinal inflammation has been reported in patients with IBD treated with secukinumab or brodalumab thus increasing disease severity and has been responsible for drug-induced IBD (41). Similarly, various IL-17 biologics have been associated with increased numbers of *Candida* or upper respiratory tract infections in numerous different patient groups, further increasing patient morbidity (40, 42). Psoriasis patients treated with brodalumab have experienced depression/anxiety linked with suicidal thoughts (43). It is well accepted within the field that stringent pharmacovigilance measures are required to ascertain drug safety and adverse risk events.

The short antigen recognition sequence (approximately 6 times shorter) of Ab-IPL-IL-17<sup>TM</sup> offers a significant clinical advantage over other known anti-IL17 monoclonal antibodies, by reducing the incidence of non-specific binding that can result from longer amino acid sequences (44). Moreover, short peptide sequences also offer lower production costs and manufacturing advantages when compared with large protein targets. Of potential clinical importance, Ab-IPL-IL-17<sup>TM</sup> displays, in mice, equivalent efficacy as reference and gold-standard current treatment commercially available anti-IL-17 neutralising antibodies, but crucially displays reduced

immunogenicity and haematological side effects that are major issues currently faced by patients (8, 13).

In conclusion, we have identified the bioactive sequence of IL-17 that is responsible for driving inflammation, and which has conserved sequence homology in mice and humans. Using this unique sequence, we have generated a specific cross-species neutralising antibody allowing discovery *in vitro* and pre-clinical rodent assessment, as well as having the potential to be translated directly into clinic as a new therapy. Crucially Ab-IPL-IL-17™ has no immunogenicity, lymphocytosis or thrombocytopenia properties, highlighting its clinical superiority over current therapies, including secukinumab. Future studies and clinical trials will need to address the varying requirements of Ab-IPL-IL17™ as an alternative biological therapy for treating patients with IMIDs.

## **MATERIALS AND METHODS**

### **Animals**

Experiments were carried out in 8-12-week-old male CD-1 mice according to the guidelines for the safe use and care of experimental animals in accordance with the Italian D.L.no.116 of 27 January 1992 (500/2020-PR, 507/2022-PR) and associated guidelines in the European Communities Council (86/609/ECC and 2010/63/UE) including the 3Rs concept (45). Animals were housed with *ad libitum* access to food and water and maintained on a 12 h light/dark cycle. Experimental study groups were randomised and blinded. All procedures were carried out to minimize the number of animals used (n = 5-7 per group) and their suffering.

### **Air pouch**

Dorsal air pouches were prepared by injection of 2.5 ml of air on day 0 and day 3 in CD-1 mice, as previously described (25). On day 6, mice received 0.25 ml of one of the following treatments diluted in 0.5% carboxymethyl cellulose (CMC, Sigma-Aldrich): i) vehicle, CMC alone; ii) IL-17 (1 µg); iii) nIL-17™ (1 µg); iv) IL-17 (1 µg) plus MAB421 or Ab-IPL-IL-17™ (10 µg); v) IL-17 (1 µg) plus anti-JE (10 µg, MAB479, R&D System); vi) IL-17 (1 µg) plus anti-KC (10 µg, MAB453, R&D System). Mice were sacrificed after 24 h, and lavage fluids were recovered, and centrifuged at 220 g for 10 min at 4 °C. Cell pellets and inflammatory exudates were banked for subsequent analysis. Collected and measured to evaluate the level of inflammatory cyto-chemokines. The route, timing, and frequency of administration as well as the selected dosages of tested compounds were selected according to updated literature (12, 16). Cell number was determined by TC20 automated cell counter (Bio-Rad) using Bio-Rad's TC20 automated cell counter uses disposable slides, TC20 trypan blue dye (0.4% trypan blue dye w/v in 0.81% sodium chloride and 0.06% potassium phosphate dibasic solution, Sigma-Aldrich) and a CCD camera to count cells based on the analyses of capture images (25).

### **AIA model**

Animal studies were regulated by the Animals (Scientific Procedures) Act 1986 of the United Kingdom and performed under appropriate Personal Project License. Approval was granted by the University of Birmingham's Animal Welfare and Ethical Review Body and all ethical guidelines were adhered to whilst carrying out this study. Eight-week-old male, C57Bl/6J wild type (WT) mice were purchased from Charles River and were maintained in a specific pathogen free facility, with free access to food and water. Environmental conditions were: 21 ± 2 °C, 55 ± 10% relative humidity and a 12 h light-dark cycle. Mice were immunised with methylated bovine serum albumin (mBSA, 10 µg subcutaneous [s.c.], Sigma-Aldrich) in complete Freund's adjuvant (CFA, Thermofisher scientific, Milan, Italy) (46). On day 21, monoarthritis was induced by intraarticular

injection of mBSA (100 µg) into the knee. Mice were treated therapeutically at 24 h or 72 h post disease onset by intraperitoneal (i.p.) injection with 50 µg of either infliximab (anti- TNF-α) or a neutralising antibody to IL-17 (Ab-IPL-IL-17™). Joint thickness (mm) was measured by callipers daily for up to 7 days. Data are expressed as a percentage change from baseline measurement taken on day 21 or area under the curve (AUC).

### **Human blood samples**

Whole blood was collected in EDTA coated vacutainers from healthy donors and IBD patients (47-49) with written informed consent and approval from the University of Birmingham Local Ethical Review Committee (ERN\_18-0382; RG\_21-009). An equal proportion of male and female donors were used with an age range between 22-70.

### **Statistical analysis**

Statistical analysis complies with the international recommendations on experimental design and analysis in pharmacology and data sharing and presentation in preclinical pharmacology (30, 50). Data are presented as mean ± S.D. or median ± interquartile range. Normality was tested by xxx prior to analysis with one or two-way ANOVA followed by Bonferroni's or Dunnett's for multiple comparisons, where  $P \leq 0.05$  was deemed significant. Animal weight was used for randomization and group allocation to reduce unwanted sources of variations by data normalization. No animals and related *ex vivo* samples were excluded from the analysis. *In vivo* study was carried out to generate groups of equal size ( $n = 5-7$  of independent values), using randomization and blinded analysis.

### **Experimental Procedures and Materials**

Murine and human cell lines and culture, *ex vivo* whole blood assay, Elisa-based binding assay, transwell migration assay, *ex vivo* analysis, haematological investigations, western blot analysis, flow cytometry, synthesis of a novel IL-17 neutralising antibody, immunization and fusion protocols and computational studies are described in Supplementary Materials and Methods.

### **Data, Materials, and Software Availability**

All data associated with this study are present in the paper or the Supplementary Materials. Requests for reagents (antibodies and other proteins) should be directed to the corresponding authors and will be made available after completion of a material transfer agreement with the University of Naples.

### **Funding and Acknowledgments**

This work was partially supported by grants from New.Fa.Dem. S.R.L. (Grant Number PG/2021/0072677). A.J.I. was supported by Birmingham Fellowship. A.A.M. was supported by a King Khalid University funded PhD Scholarship (57875). F.R. and A.Sa. were supported by the University of Naples Federico II PhD scholarship in Pharmaceutical Sciences and Dompé Farmaceutici S.p.A fellowship for PhD program in "Nutraceuticals, functional foods and human health" respectively (University of Naples Federico II). J.E.M. was supported by a Medical Research Council (MRC) Industry Case Studentship (MR/P016154/1). M.W. and H.M.M. were supported by a MRC Project Grant (MR/T028025/1). The authors would like to thank ProteoGenix (France) for excellent technical assistance and scientific discussions on the manuscript. The

views expressed in this publication are those of the author(s) and not necessarily those of the New.Fa.Dem SRL.

## References

1. Gaffen SL, Jain R, Garg AV, *et al.* The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nat Rev Immunol* 2014;**14**(9):585-600. doi:10.1038/nri3707
2. Ishigame H, Kakuta S, Nagai T, *et al.* Differential roles of interleukin-17A and -17F in host defense against mucocutaneous bacterial infection and allergic responses. *Immunity* 2009;**30**(1):108-19. doi:10.1016/j.immuni.2008.11.009
3. Miossec P, Kolls JK. Targeting IL-17 and TH17 cells in chronic inflammation. *Nat Rev Drug Discov* 2012;**11**(10):763-76. doi:10.1038/nrd3794
4. Maione F. Commentary: IL-17 in Chronic Inflammation: From Discovery to Targeting. *Front Pharmacol* 2016;**7**:250. doi:10.3389/fphar.2016.00250
5. Mills KHG. IL-17 and IL-17-producing cells in protection versus pathology. *Nat Rev Immunol* 2023;**23**(1):38-54. doi:10.1038/s41577-022-00746-9
6. Raucci F, Mansour AA, Casillo GM, *et al.* Interleukin-17A (IL-17A), a key molecule of innate and adaptive immunity, and its potential involvement in COVID-19-related thrombotic and vascular mechanisms. *Autoimmun Rev* 2020;**19**(7):102572. doi:10.1016/j.autrev.2020.102572
7. Berry SPD, Dossou C, Kashif A, *et al.* The role of IL-17 and anti-IL-17 agents in the immunopathogenesis and management of autoimmune and inflammatory diseases. *Int Immunopharmacol* 2022;**102**:108402. doi:10.1016/j.intimp.2021.108402
8. Baker KF, Isaacs JD. Novel therapies for immune-mediated inflammatory diseases: What can we learn from their use in rheumatoid arthritis, spondyloarthritis, systemic lupus erythematosus, psoriasis, Crohn's disease and ulcerative colitis? *Ann Rheum Dis* 2018;**77**(2):175-87. doi:10.1136/annrheumdis-2017-211555
9. Liu S, Song X, Chrnyk BA, *et al.* Crystal structures of interleukin 17A and its complex with IL-17 receptor A. *Nat Commun* 2013;**4**:1888. doi:10.1038/ncomms2880
10. Gaffen SL. Recent advances in the IL-17 cytokine family. *Curr Opin Immunol* 2011;**23**(5):613-9. doi:10.1016/j.coi.2011.07.006
11. Zrioual S, Toh ML, Tournadre A, *et al.* IL-17RA and IL-17RC receptors are essential for IL-17A-induced ELR+ CXC chemokine expression in synoviocytes and are overexpressed in rheumatoid blood. *J Immunol* 2008;**180**(1):655-63. doi:10.4049/jimmunol.180.1.655
12. Raucci F, Iqbal AJ, Saviano A, *et al.* IL-17A neutralizing antibody regulates monosodium urate crystal-induced gouty inflammation. *Pharmacol Res* 2019;**147**:104351. doi:10.1016/j.phrs.2019.104351
13. Álvarez-Coiradas E, Munteanu CR, Díaz-Sáez L, *et al.* Discovery of novel immunopharmacological ligands targeting the IL-17 inflammatory pathway. *Int Immunopharmacol* 2020;**89**(Pt A):107026. doi:10.1016/j.intimp.2020.107026
14. Yao Z, Fanslow WC, Seldin MF, *et al.* Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity* 1995;**3**(6):811-21. doi:10.1016/1074-7613(95)90070-5
15. Li X, Bechara R, Zhao J, *et al.* IL-17 receptor-based signaling and implications for disease. *Nat Immunol* 2019;**20**(12):1594-602. doi:10.1038/s41590-019-0514-y
16. Raucci F, Saviano A, Casillo GM, *et al.* IL-17-induced inflammation modulates the mPGES-1/PPAR- $\gamma$  pathway in monocytes/macrophages. *Br J Pharmacol* 2022;**179**(9):1857-73. doi:10.1111/bph.15413
17. Lamiable A, Thévenet P, Rey J, *et al.* PEP-FOLD3: faster de novo structure prediction for linear peptides in solution and in complex. *Nucleic Acids Res* 2016;**44**(W1):W449-54. doi:10.1093/nar/gkw329
18. Micsonai A, Wien F, Kernya L, *et al.* Accurate secondary structure prediction and fold recognition for circular dichroism spectroscopy. *Proc Natl Acad Sci U S A* 2015;**112**(24):E3095-103. doi:10.1073/pnas.1500851112

19. Ha HL, Wang H, Pisitkun P, *et al.* IL-17 drives psoriatic inflammation via distinct, target cell-specific mechanisms. *Proc Natl Acad Sci U S A* 2014;**111**(33):E3422-31. doi:10.1073/pnas.1400513111
20. Jovanovic DV, Di Battista JA, Martel-Pelletier J, *et al.* IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. *J Immunol* 1998;**160**(7):3513-21.
21. Erbel C, Akhavanpoor M, Okuyucu D, *et al.* IL-17A influences essential functions of the monocyte/macrophage lineage and is involved in advanced murine and human atherosclerosis. *J Immunol* 2014;**193**(9):4344-55. doi:10.4049/jimmunol.1400181
22. Wang R, Campbell S, Amir M, *et al.* Genetic and pharmacological inhibition of the nuclear receptor ROR $\alpha$  regulates T(H)17 driven inflammatory disorders. *Nat Commun* 2021;**12**(1):76. doi:10.1038/s41467-020-20385-9
23. Griffin GK, Newton G, Tarrío ML, *et al.* IL-17 and TNF- $\alpha$  sustain neutrophil recruitment during inflammation through synergistic effects on endothelial activation. *J Immunol* 2012;**188**(12):6287-99. doi:10.4049/jimmunol.1200385
24. Maione F, Paschalidis N, Mascolo N, *et al.* Interleukin 17 sustains rather than induces inflammation. *Biochem Pharmacol* 2009;**77**(5):878-87. doi:10.1016/j.bcp.2008.11.011
25. Maione F, Iqbal AJ, Raucci F, *et al.* Repetitive Exposure of IL-17 Into the Murine Air Pouch Favors the Recruitment of Inflammatory Monocytes and the Release of IL-16 and TREM-1 in the Inflammatory Fluids. *Front Immunol* 2018;**9**:2752. doi:10.3389/fimmu.2018.02752
26. Miyamoto M, Prause O, Sjöstrand M, *et al.* Endogenous IL-17 as a mediator of neutrophil recruitment caused by endotoxin exposure in mouse airways. *J Immunol* 2003;**170**(9):4665-72. doi:10.4049/jimmunol.170.9.4665
27. Roussel L, Houle F, Chan C, *et al.* IL-17 promotes p38 MAPK-dependent endothelial activation enhancing neutrophil recruitment to sites of inflammation. *J Immunol* 2010;**184**(8):4531-7. doi:10.4049/jimmunol.0903162
28. Pelletier M, Maggi L, Micheletti A, *et al.* Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood* 2010;**115**(2):335-43. doi:10.1182/blood-2009-04-216085
29. Cristiano C, Volpicelli F, Lippiello P, *et al.* Neutralization of IL-17 rescues amyloid- $\beta$ -induced neuroinflammation and memory impairment. *Br J Pharmacol* 2019;**176**(18):3544-57. doi:10.1111/bph.14586
30. Vellecco V, Saviano A, Raucci F, *et al.* Interleukin-17 (IL-17) triggers systemic inflammation, peripheral vascular dysfunction, and related prothrombotic state in a mouse model of Alzheimer's disease. *Pharmacol Res* 2023;**187**:106595. doi:10.1016/j.phrs.2022.106595
31. Beringer A, Noack M, Miossec P. IL-17 in Chronic Inflammation: From Discovery to Targeting. *Trends Mol Med* 2016;**22**(3):230-41. doi:10.1016/j.molmed.2016.01.001
32. McGettrick HM, Smith E, Filer A, *et al.* Fibroblasts from different sites may promote or inhibit recruitment of flowing lymphocytes by endothelial cells. *Eur J Immunol* 2009;**39**(1):113-25. doi:10.1002/eji.200838232
33. Schoppmeyer R, van Steen ACI, Kempers L, *et al.* The endothelial diapedesis synapse regulates transcellular migration of human T lymphocytes in a CX3CL1- and SNAP23-dependent manner. *Cell Rep* 2022;**38**(3):110243. doi:10.1016/j.celrep.2021.110243
34. Spindeldreher S, Maillère B, Correia E, *et al.* Secukinumab Demonstrates Significantly Lower Immunogenicity Potential Compared to Ixekizumab. *Dermatol Ther (Heidelb)* 2018;**8**(1):57-68. doi:10.1007/s13555-018-0220-y
35. Balato A, Scala E, Balato N, *et al.* Biologics that inhibit the Th17 pathway and related cytokines to treat inflammatory disorders. *Expert Opin Biol Ther* 2017;**17**(11):1363-74. doi:10.1080/14712598.2017.1363884
36. Corneth OB, Mus AM, Asmawidjaja PS, *et al.* Absence of interleukin-17 receptor a signaling prevents autoimmune inflammation of the joint and leads to a Th2-like phenotype in collagen-induced arthritis. *Arthritis Rheumatol* 2014;**66**(2):340-9. doi:10.1002/art.38229
37. Lemos HP, Grespan R, Vieira SM, *et al.* Prostaglandin mediates IL-23/IL-17-induced neutrophil migration in inflammation by inhibiting IL-12 and IFN $\gamma$  production. *Proc Natl Acad Sci U S A* 2009;**106**(14):5954-9. doi:10.1073/pnas.0812782106

38. Hwang SY, Kim JY, Kim KW, *et al.* IL-17 induces production of IL-6 and IL-8 in rheumatoid arthritis synovial fibroblasts via NF-kappaB- and PI3-kinase/Akt-dependent pathways. *Arthritis Res Ther* 2004;**6**(2):R120-8. doi:10.1186/ar1038
39. Fauny M, Moulin D, D'Amico F, *et al.* Paradoxical gastrointestinal effects of interleukin-17 blockers. *Ann Rheum Dis* 2020;**79**(9):1132-8. doi:10.1136/annrheumdis-2020-217927
40. Eshwar V, Kamath A, Shastry R, *et al.* A Review of the Safety of Interleukin-17A Inhibitor Secukinumab. *Pharmaceuticals (Basel)* 2022;**15**(11). doi:10.3390/ph15111365
41. Darch KM, Holland TL, Spelman LJ. Secukinumab-Induced Inflammatory Bowel Disease in a Patient Treated for Chronic Plaque Psoriasis and Psoriatic Arthritis: A Case Report and Review of the Role of Novel Biologic Agents Targeting the p19 Subunit of IL-23. *Case Rep Med* 2020;**2020**:9404505. doi:10.1155/2020/9404505
42. Feng Y, Zhou B, Wang Z, *et al.* Risk of Candida Infection and Serious Infections in Patients with Moderate-to-Severe Psoriasis Receiving Biologics: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Int J Clin Pract* 2022;**2022**:2442603. doi:10.1155/2022/2442603
43. Schmidt C. Suicidal thoughts end Amgen's blockbuster aspirations for psoriasis drug. *Nat Biotechnol* 2015;**33**(9):894-5. doi:10.1038/nbt0915-894b
44. Shores LS, Kelly SH, Hainline KM, *et al.* Multifactorial Design of a Supramolecular Peptide Anti-IL-17 Vaccine Toward the Treatment of Psoriasis. *Front Immunol* 2020;**11**:1855. doi:10.3389/fimmu.2020.01855
45. Kilkenny C, Browne W, Cuthill IC, *et al.* Animal research: reporting in vivo experiments: the ARRIVE guidelines. *Br J Pharmacol* 2010;**160**(7):1577-9. doi:10.1111/j.1476-5381.2010.00872.x
46. Asquith DL, Miller AM, McInnes IB, *et al.* Animal models of rheumatoid arthritis. *Eur J Immunol* 2009;**39**(8):2040-4. doi:10.1002/eji.200939578
47. Krautter F, Recio C, Hussain MT, *et al.* Characterisation of endogenous Galectin-1 and -9 expression in monocyte and macrophage subsets under resting and inflammatory conditions. *Biomed Pharmacother* 2020;**130**:110595. doi:10.1016/j.biopha.2020.110595
48. Myrianthefs P, Karatzas S, Venetsanou K, *et al.* Seasonal variation in whole blood cytokine production after LPS stimulation in normal individuals. *Cytokine* 2003;**24**(6):286-92. doi:10.1016/j.cyto.2003.08.005
49. Papandreou V, Kavrochorianou N, Katsoulas T, *et al.* Adrenergic Effect on Cytokine Release After Ex Vivo Healthy Volunteers' Whole Blood LPS Stimulation. *Inflammation* 2016;**39**(3):1069-75. doi:10.1007/s10753-016-0338-y
50. Saviano A, Raucci F, Casillo GM, *et al.* Anti-inflammatory and immunomodulatory activity of *Mangifera indica* L. reveals the modulation of COX-2/mPGES-1 axis and Th17/Treg ratio. *Pharmacol Res* 2022;**182**:106283. doi:10.1016/j.phrs.2022.106283

## Figure Legends

**Fig. 1. Biological characterisation of a novel IL-17-derived peptide (nIL-17™).** (A) Amino acids sequence of nIL-17™ was obtained after a study of primary structures of both mouse/human IL-17A and IL-17F. (B) To assess the biological activity of nIL-17™ peptide, IL-6 production was evaluated in NIH-3T3 cell supernatants following 24 h of incubation in presence of either IL-17A protein (50 ng/ml), IL-17F protein (50 ng/ml), IL-17A/F heterodimer (50 ng/ml), nIL-17™ (50 ng/ml), nIL-17™ (50 ng/ml) with terminal NH<sub>2</sub> sequence, denatured (-DN) form or "scrambled" (-SC) sequence (both at 50 ng/ml). (C-D) Whole cell lysates from NIH-3T3 cells stimulated with IL-17 or nIL-17™ (50 ng/ml) were analysed, by western blot, for IL-17RA (~120 kDa), IL-17RC (~110 kDa), Act-1 (~72 kDa), NFκB (~65 kDa) and actin (~42 kDa) expression. Representative western blot images are shown from three pooled experiments with similar results. (E) To evaluate the binding interaction of nIL-17™ with IL-17RA and RC, biotinylated IL-17 and nIL-17™ (0-750 ng/ml) were co-incubated for 30 min with IL-17RA-Fc or IL-17RC-Fc prior to fluorescence being measured. (B-E) Data are presented as mean ± S.D. of n = 3 independent experiments. (F-G) Macrophages, derived from primary human CD14<sup>+</sup> monocytes, were stimulated with LPS and IFN-γ (M1 stimuli) for 16 h. Following differentiation, M1 macrophages were treated with IL-17 vehicle, IL-17, or nIL-17™ (100 ng/ml) for 24 h.

Supernatants from all experimental conditions were assayed by Elisa for (F) IL-6, and (G) TNF- $\alpha$ . (H) Transwell chemotaxis assay was employed to determine the chemotactic activity of nIL-17<sup>TM</sup>. M199 media (final volume 700  $\mu$ l) was added to the bottom well of a Transwell-24 permeable support with 3.0  $\mu$ m pores with IL-17 (10-500 ng/ml), nIL-17<sup>TM</sup> (10-500 ng/ml), or formyl-methionyl-leucyl-phenylalanine (fMLP; 10<sup>-6</sup> M as positive control). Neutrophils were added to the top chamber, which had a confluent stimulated (TNF- $\alpha$  and IFN- $\gamma$ ) HDBEC monolayer. (H) After 2 h of incubation at 37 °C neutrophils were collected from the bottom of the wells and quantified using flow cytometry. (F-H) Data are presented as mean  $\pm$  S.D. of n = 3 healthy donors. Statistical analysis was performed using the one-way ANOVA test followed by Bonferroni. #P  $\leq$  0.05, ##P  $\leq$  0.01, ###P  $\leq$  0.001, ####P  $\leq$  0.0001 vs vehicle group; \*P  $\leq$  0.05, \*\*P  $\leq$  0.01, \*\*\*P  $\leq$  0.001 vs IL-17s group.

**Fig. 2. nIL-17<sup>TM</sup> promotes leukocyte recruitment *in vivo* and migration *in vitro*.** To evaluate the pro-inflammatory activity of nIL-17<sup>TM</sup>, we utilised a sub-chronic model of inflammation, the dorsal air pouch. (A) Mice were treated with IL-17 vehicle (0.5% CMC), IL-17 (1  $\mu$ g/pouch), or nIL-17<sup>TM</sup> (1  $\mu$ g/pouch). (B) Total CD45<sup>+</sup> leukocyte numbers were quantified by flow cytometry. (B) Data are presented as means  $\pm$  S.D. of n = 7 mice per group. (C-E) Inflammatory supernatants obtained from pouch cavities were assayed using a Proteome Profiler cytokine array. (F) Densitometric analysis is presented as a heat map with dots indicating the most significant modulated cyto-chemokines mediators. (F) Data are presented as means  $\pm$  S.D. of positive spots from three independent experiments run each with n = 7 mice per group pooled. (G-I) To determine the impact of nIL-17<sup>TM</sup> on leukocyte adhesion and transmigration on HDBEC a static migration assay was used. HDBECs were treated with IL-17 vehicle (HCl 4 mM PBS), IL-17 (100 ng/ml) or nIL-17<sup>TM</sup> (100 ng/ml), alone or in combination with TNF- $\alpha$  (100 U/ml) for 24 h. (G) Representative images of the static adhesion assay are shown (200  $\mu$ m magnification). PBMCs were added for 20 min on stimulated HDBEC, followed by washing to remove all non-adherent cells. Phase bright PBMCs were considered (H) adherent (red arrow), whereas phase-dark were quantified as (I) transmigrated (% of adherent cells) (orange arrow). (J-K) VCAM-1 and ICAM-1 expression on HDBECs was quantified by flow cytometry. (H-K) Data are presented as means  $\pm$  S.D. of n = 3 independent healthy donors. Statistical analysis was conducted by one or two-way ANOVA followed by Bonferroni's for multiple comparisons. #P $\leq$ 0.05, ##P $\leq$ 0.01, ###P $\leq$ 0.001, ####P $\leq$ 0.0001 vs vehicle group; \*\*\*\*P $\leq$ 0.0001 vs IL-17 group; \*\*\*P $\leq$ 0.001 vs TNF- $\alpha$  group.

**Fig. 3. Biological characterisation of a novel IL-17 neutralising antibody (Ab-IPL-IL-17<sup>TM</sup>).** (A) To assess the biological activity of Ab-IPL-IL-17<sup>TM</sup>, IL-6 production was evaluated in NIH-3T3 cell supernatants following 24 h treatment with IL-17 (50 ng/ml) or nIL-17<sup>TM</sup> (50 ng/ml) alone or in combination with Ab-IPL-IL-17<sup>TM</sup> (75-750 ng/ml). (B) To analyse the neutralisation effect of Ab-IPL-IL-17<sup>TM</sup> on IL-17/IL-17Rs interactions, biotinylated IL-17 (EC<sub>50</sub> concentrations) and Ab-IPL-IL-17<sup>TM</sup> (0-750 ng/ml) complex was co-incubated for 30 min with IL-17RA-Fc or IL-17RC-Fc prior to fluorescence being measured. (A-B) Data are presented as mean  $\pm$  S.D. of n = 3 independent experiments. (C-D) Macrophages, derived from primary human CD14<sup>+</sup> monocytes, were stimulated with LPS and IFN- $\gamma$  (M1-stimuli) over 16 h. Following differentiation, cells were treated with IL-17 vehicle, IL-17 (100 ng/ml) alone or in combination with Ab-IPL-IL-17<sup>TM</sup> (10  $\mu$ g/ml) for 24 h. Supernatants from all experimental conditions were assayed by Elisa for (C) IL-6, and (D) TNF- $\alpha$ . (E) For the transwell chemotaxis assay, neutrophils were added to the top chamber which had a confluent stimulated (TNF- $\alpha$  and IFN- $\gamma$ ) HDBEC monolayer. (E) Chemotactic migration to IL-17 (500 ng/ml) alone or in combination with Ab-IPL-IL-17<sup>TM</sup> (10  $\mu$ g/ml) was quantified using flow cytometry. (C-E) Data are presented as means  $\pm$  S.D. of n = 3 independent healthy donors. (F) For *in vivo* experiment, mice were treated with IL-17 vehicle (0.5% CMC), IL-17 (1  $\mu$ g/pouch) alone or in co-administration with Ab-IPL-IL-17<sup>TM</sup> (10  $\mu$ g/ml), and thereafter total CD45<sup>+</sup> leukocyte numbers were quantified by flow cytometry. (F) Data are presented as means  $\pm$  S.D. of n = 7 mice per group. (G) Inflammatory supernatants obtained from the pouch cavities were assayed using a Proteome Profiler cytokine



array. Densitometric analyses is presented as a heat map indicating the most significant modulated cyto-chemokines mediators. (G) Data are presented as means  $\pm$  S.D. of positive spots of three separate independent experiments run each with  $n = 7$  mice per group pooled. (H-J) HDBECs were treated with IL-17 vehicle (HCl 4 mM PBS), IL-17 (100 ng/ml) plus TNF- $\alpha$  (100 U/ml), alone or in combination with Ab-IPL-IL-17<sup>TM</sup> (10 $\mu$ g/ml) for 24 h. Phase bright PBMCs were considered (H) adherent (red arrow), whereas phase-dark were quantified as (I) transmigrated (% of adherent cells) (orange arrow). (J) Representative images of the static adhesion assay are shown (200  $\mu$ m magnification). (K-L) VCAM-1, and ICAM-1 expression on HDBECs was quantified by flow cytometry. (H-L) Data are presented as means  $\pm$  S.D. of  $n = 3$  independent healthy donors. Statistical analysis was conducted by one or two-way ANOVA followed by Bonferroni's for multiple comparisons.  $^{##}P \leq 0.01$ ,  $^{###}P \leq 0.001$ ,  $^{####}P \leq 0.0001$  vs vehicle group;  $^{*}P \leq 0.05$ ,  $^{**}P \leq 0.01$ ,  $^{***}P \leq 0.0001$  vs IL-17 group;  $^{§§}P \leq 0.01$ ,  $^{§§§}P \leq 0.001$ ,  $^{§§§§}P \leq 0.0001$  vs nIL-17<sup>TM</sup> group;  $^{*}P \leq 0.05$ ,  $^{**}P \leq 0.01$ ,  $^{***}P \leq 0.0001$  vs IL-17 + TNF- $\alpha$  group.

**Fig. 4. Ab-IPL-IL-17<sup>TM</sup> displays a protective profile in murine preclinical models of immune-mediated inflammatory diseases.** To assess the neutralising activity of Ab-IPL-IL-17<sup>TM</sup>, CD-1 mice were injected i.p. with 100  $\mu$ g/mouse of Ab-IPL-IL-17<sup>TM</sup>, MAB421, secukinumab or bimekizumab as positive controls. After 30 min, an i.p. injection of 10  $\mu$ g/mouse of IL-17A, IL-17F or IL-17A/F heterodimer was administered. After 2 h blood was collected by intracardiac puncture and serum levels of (A) IL-17A, (B) IL-17F or (C) IL-17A/F were quantified by Elisa. (D-G) For the evaluation of immunogenic effects CD-1 mice were injected i.p. with 100  $\mu$ g of IgG1 isotype antibody (vehicle) or IL-17 neutralising antibodies (secukinumab, bimekizumab, MAB421 or Ab-IPL-IL-17<sup>TM</sup>). In the selected time-point (2 h, 24 h, 72 h, 7 days, 14 days and 21 days) (D) total IgG, (E) IgG1, (F) lymphocytes and (G) platelets levels were determined by Elisa and haematological blood count test, respectively. Data are presented as mean  $\pm$  S.D. for  $n = 5$  mice per group. Statistical analysis was conducted by one or two-way ANOVA followed by Bonferroni's for multiple comparisons.  $^{*}P \leq 0.05$ ,  $^{**}P \leq 0.01$ ,  $^{***}P \leq 0.001$  vs IL-17 group;  $^{#}P \leq 0.05$ ,  $^{##}P \leq 0.01$ ,  $^{###}P \leq 0.001$ ,  $^{####}P \leq 0.0001$  vs vehicle group; (in red refers to secukinumab and light blue refers to MAB421 respectively).

**Fig. 5. Ab-IPL-IL-17<sup>TM</sup> displays a protective profile in human preclinical models of immune-mediated inflammatory diseases.** Monoarthritic mice (AIA group) were therapeutically administered Ab-IPL-IL-17<sup>TM</sup> or infliximab (anti-TNF- $\alpha$ ) on day 1 and 3. Joint inflammation was scored daily and expressed as (A) percentage of baseline joint thickness or (B) AUC. Flow cytometry analysis was employed to determine *in situ* neutrophil and monocyte levels. At 7-day time-point, ankle joints were digested, and total cells were gated, followed by single cells, before the identifications of CD45. CD45<sup>+</sup> cells were plotted to identify the % of CD11b<sup>+</sup>/ LY6C<sup>+</sup>/ LY6G<sup>+</sup> as neutrophils (C) and CD11b<sup>+</sup>/ F4/80<sup>low</sup>/ LY6C<sup>+</sup> as monocytes (D). Data are presented as mean  $\pm$  S.D. for  $n = 5-6$  mice per group. Statistical analysis was conducted by one or two-way ANOVA followed by Dunnett post-test.  $^{*}P \leq 0.05$ ,  $^{**}p \leq 0.01$  vs AIA group. (E-F) Fibroblasts from resolving arthritis or RA patients were treated with IL-17 (10 ng/ml) and TNF- $\alpha$  (100 U/ml) alone or in combination with MAB317 or Ab-IPL-IL-17<sup>TM</sup> (10  $\mu$ g/ml). Secretion of (E) IL-6 or (F) IL-8 were measured by ELISA. Data are median  $\pm$  interquartile ranges (min 25%, max 75%) for  $n = 3$  independent donors. (E-F) Statistical analysis was conducted by one-way ANOVA followed by Bonferroni's for multiple comparisons.  $^{####}P \leq 0.001$ ,  $^{#####}P \leq 0.0001$  vs own vehicle group;  $^{**}P \leq 0.01$  vs own IL-17 + TNF- $\alpha$ ;  $^{*}P \leq 0.05$  vs Resolving IL-17 + TNF- $\alpha$ . (G) Human whole blood from IBD patients was treated with or without Ab-IPL-IL-17<sup>TM</sup> (10  $\mu$ g/ml) for 4 h, after which serum IL-17 levels were assessed by Elisa assay. Data are median  $\pm$  interquartile ranges (min 25%, max 75%) for  $n = 6$  independent donors. Statistical analysis was conducted by one-way ANOVA followed by Bonferroni's for multiple comparisons.  $^{#}P \leq 0.05$  vs vehicle group.

	Vehicle (n=5)	Ab-IPL-IL-1 7™ (n=5)	MAB421 (n=5)	Secukinumab (n=5)	Bimekizumab (n=5)
<b>WBC<sup>††</sup></b>					
<b>10<sup>3</sup>/μL</b>					
2 h	3.60±1.43	3.78±1.29	3.84±1.30	3.96±1.19	3.90±0.91
24 h	3.52±0.91	4.06±1.70	4.08±1.22	4.44±1.88	4.60±1.70
72 h	3.38±1.15	4.48±1.99	4.56±2.25	5.10±2.34	4.84±2.49
7 d	3.60±1.29	4.02±1.90	4.08±1.77	4.74±1.30	5.08±1.37
14 d	3.60±0.83	3.88±0.81	3.70±1.30	3.08±0.86	3.00±0.68
21 d	3.34±0.94	3.44±1.40	3.30±1.58	3.280±1.47	3.32±1.18
<b>MID<sup>††</sup></b>					
<b>10<sup>3</sup>/μL</b>					
2 h	0.34±0.23	0.38±0.19	0.36±0.23	0.38±0.31	0.36±0.24
24 h	0.34±0.21	0.48±0.19	0.46±0.13	0.44±0.25	0.42±0.31
72 h	0.38±0.15	0.56±0.23	0.54±0.21	0.40±0.25	0.42±0.22
7 d	0.34±0.25	0.46±0.09	0.42±0.26	0.40±0.16	0.46±0.18
14 d	0.38±0.19	0.44±0.11	0.40±0.16	0.38±0.13	0.40±0.10
21 d	0.34±0.22	0.36±0.11	0.36±0.17	0.34±0.15	0.32±0.16
<b>GRA<sup>††</sup></b>					
<b>10<sup>3</sup>/μL</b>					
2 h	0.20±0.16	0.22±0.19	0.20±0.16	0.22±0.16	0.24±0.11
24 h	0.20±0.10	0.26±0.15	0.26±0.13	0.24±0.11	0.22±0.16
72 h	0.22±0.11	0.38±0.08	0.34±0.11	0.30±0.16	0.30±0.10
7 d	0.20±0.10	0.28±0.08	0.28±0.13	0.24±0.13	0.26±0.11
14 d	0.18±0.08	0.26±0.11	0.24±0.13	0.20±0.10	0.20±0.12
21 d	0.20±0.10	0.24±0.05	0.24±0.11	0.20±0.10	0.22±0.13
<b>RBC<sup>††</sup></b>					
<b>10<sup>6</sup>/μL</b>					
2 h	5.99±1.29	5.74±1.22	5.56±1.12	5.57±1.18	5.84±1.62
24 h	6.09±1.203	5.96±1.05	5.66±1.21	4.48±1.11	4.63±0.99
72 h	6.12±1.02	6.30±0.78	6.29±1.19	3.7±1.05 *	3.79±1.02*
7 d	6.14±1.19	6.29±0.95	6.09±0.66	4.10±0.78	4.18±0.93
14 d	6.12±1.03	6.47±1.00	6.12±0.46	5.98±1.42	5.81±1.41
21 d	6.15±0.98	6.41±0.96	6.27±0.54	6.25±1.18	6.37±1.11

<b>HGB<sup>††</sup></b>						
<b>g/dL</b>						
2 h	9.84±2.22	10.58±1.55	10.10±1.87	9.92±1.78	9.88±1.02	
24 h	10.08±1.74	10.72±1.50	10.26±1.62	8.88±1.65	9.08±1.55	
72 h	10.20±1.73	10.84±1.56	10.26±1.71	6.92±1.53*	7.10±1.38*	
7 d	10.32±1.366	10.74±1.03	10.20±1.17	7.76±1.31	8.10±1.33	
14 d	10.28±1.593	10.96±1.23	10.34±1.30	9.480±1.36	9.06±1.84	
21 d	10.22±1.897	10.90±1.06	10.42±1.08	10.16±1.52	10.06±1.27	

<b>HCT<sup>††</sup> %</b>						
2 h	30.24±6.13	29.84±4.21	28.62±3.68	29.56±2.26	29.96±2.29	
24 h	30.30±5.25	30.24±3.57	28.98±3.97	28.86±1.80	29.56±2.16	
72 h	31.00±5.00	31.64±3.64	30.28±4.38	21.30±5.47**	22.04±4.37**	
7 d	30.58±4.76	31.46±2.97	29.58±4.05	22.94±4.68*	22.48±3.74*	
14 d	30.70±5.35	31.86±1.87	30.48±1.80	27.88±2.63	28.36±3.19	
21 d	31.00 ±4.61	31.88±2.12	31.16±2.23	29.04±2.30	29.20±2.70	

<b>MCV<sup>††</sup> fL</b>						
2 h	62.96±10.27	60.62±8.00	60.76±7.5	61.86±8.95	61.22±5.70	
24 h	64.16±10.23	62.32±8.25	61.22±6.33	63.60±8.78	62.32±10.33	
72 h	64.24±11.81	62.98±8.30	61.34±8.01	81.12±8.84	80.98±9.17	
7 d	63.06±9.67	62.60±9.27	62.00±6.18	71.98±8.64	72.78±8.77	
14 d	63.12±7.37	62.62±8.51	60.76±5.23	63.46±8.13	61.10±9.83	
21 d	64.10±8.72	63.18±8.74	62.36±4.85	62.56±7.90	62.92±6.53	

<b>MCH<sup>††</sup> pg</b>						
2 h	16.88±1.83	17.48±1.32	17.52±1.06	17.58±1.42	16.98±1.38	
24 h	17.28±2.25	17.32±1.75	17.90±1.38	19.10±1.88	18.92±1.42	
72 h	17.40±2.02	17.70±1.44	17.56±1.12	21.78±4.20*	21.46±2.04*	
7 d	17.42±1.72	17.54±1.19	17.60±0.62	21.14±4.82	21.22±3.74	
14 d	17.76±1.77	17.74±1.18	17.44±0.70	17.50±1.09	17.48±1.38	
21 d	17.04±1.66	17.08±1.18	17.40±0.95	17.62±1.05	17.96±1.57	

<b>MHCH<sup>††</sup></b>						
<b>g/dL</b>						
2 h	30.48±3.57	29.94±3.12	30.76±4.19	30.60±4.27	30.00±3.06	
24 h	30.80±3.80	29.52±3.76	30.74±3.74	31.54±4.15	30.70±3.18	
72 h	31.14±3.94	30.66±3.54	30.28±4.05	30.90±6.72	30.62±5.57	
7 d	30.64±3.74	29.99±2.86	30.50±5.29	29.50±5.03	29.36±5.05	
14 d	30.82±3.00	30.72±2.61	30.34±4.24	29.64±4.38	29.96±4.45	
21 d	30.46±4.04	30.18±3.55	30.00±4.85	30.34±4.54	30.50±4.52	

<b>RDW<sup>††</sup> %</b>						
2 h	17.94±2.17	18.08±2.84	18.06±2.47	18.14±1.87	18.82±1.75	
24 h	17.72±2.03	18.04±2.91	18.54±1.56	18.24±1.12	18.42±1.18	
72 h	18.08±2.61	16.90±2.08	16.96±1.55	16.86±3.47	17.12±2.05	
7 d	18.30±2.72	17.94±1.94	17.88±1.33	18.38±1.90	18.14±3.00	
14 d	18.06±3.03	17.86±1.76	17.76±1.90	18.06±2.06	18.26±2.28	

<b>21 d</b>	18.40±2.50	17.88±1.94	17.60±0.58	18.34±1.33	18.02±1.34
<b>MPV<sup>††</sup> fL</b>					
<b>2 h</b>	4.88±0.64	4.74±0.56	4.66±0.50	4.54±0.5771	4.64±0.86
<b>24 h</b>	4.92±0.57	4.82±0.53	4.96±0.68	5.16±0.8764	5.00±1.12
<b>72 h</b>	4.98±0.52	4.96±0.53	5.08±0.58	7.80±1.733****	7.96±1.95****
<b>7 d</b>	4.88±0.62	5.00±0.67	4.84±0.54	6.58±1.242*	6.78±1.09*
<b>14 d</b>	4.90±0.50	5.12±0.67	4.98±0.57	5.86±0.5128	5.66±0.92
<b>21 d</b>	5.04±0.55	5.12±0.40	4.92±0.73	4.92±0.4207	5.02±0.76
<b>PCT<sup>††</sup> %</b>					
<b>2 h</b>	0.27±0.09	0.28±0.08	0.28±0.08	0.27±0.08	0.26±0.07
<b>24 h</b>	0.28±0.08	0.28±0.07	0.28±0.07	0.25±0.09	0.26±0.06
<b>72 h</b>	0.28±0.10	0.29±0.05	0.28±0.07	0.16±0.06	0.17±0.03
<b>7 d</b>	0.28±0.09	0.28±0.05	0.28±0.07	0.21±0.05	0.22±0.07
<b>14 d</b>	0.28±0.10	0.28±0.05	0.29±0.06	0.25±0.05	0.26±0.05
<b>21 d</b>	0.29±0.07	0.28±0.05	0.28±0.07	0.28±0.04	0.28±0.03
<b>PWD<sup>††</sup> %</b>					
<b>2 h</b>	15.98±2.63	16.24±2.34	16.28±2.31	16.30±2.15	16.80±1.42
<b>24 h</b>	15.86±2.77	16.58±2.61	16.36±2.37	16.06±2.30	16.04±1.30
<b>72 h</b>	16.02±2.52	16.30±1.98	16.36±2.25	18.02±2.61	17.94±2.99
<b>7 d</b>	15.76±2.52	16.52±2.24	16.34±2.40	15.54±2.91	16.04±2.71
<b>14 d</b>	16.04±2.68	16.72±1.73	16.28±1.98	15.98±2.03	16.02±1.63
<b>21 d</b>	16.10±2.37	16.78±1.49	16.24±2.35	16.14±1.80	16.24±1.83

<sup>††</sup>mean ± SD; WBC=White Blood Cells; MID=Minimum Inhibitory Dilution; GRA=Granulocytes; RBC=Red Blood Cells; HGB=Hemoglobin; HCT=Hematocrit; MCV=Mean Cell Volume; MCH=Mean Corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Concentration; RDW=Red blood cells Distribution Width; MPV=Mean Platelet Volume; PCT= Plateletcrit; PWD= Platelet Distribution Width.

**Table 1. Haematological parameters of vehicle, Ab-IPL-IL-17<sup>TM</sup>, MAB421 and secukinumab-treated mice.** Serum samples collected by intracardiac puncture of vehicle, Ab-IPL-IL-17<sup>TM</sup>, MAB421, secukinumab or bimekizumab (100 µg/mouse)- treated mice were assessed for haematological parameters (WBC, MID, GRA, RBC, HGB, HCT, MCV, MCH, MHCH, RDW, MPV, PCT, PWD) at indicated time-points. Results obtained were expressed as the mean ± SD. Statistical analysis was performed by using one-way ANOVA followed by Bonferroni's for multiple comparisons. \*P ≤ 0.05, \*\*P ≤ 0.01, \*\*\*\*P ≤ 0.0001 vs Vehicle group; (n = 5 mice per group).

	<b>Resolving (n=3)</b>	<b>RA (n=3)</b>
<b>Age (years) †</b>	41 (27-87)	56 (49-62)
<b>Female; number (%)</b>	2 (67)	3 (100)
<b>Symptom duration (weeks) †</b>	4 (4-7)	260 (104-1052)
<b>DAS28 ESR at baseline ††</b>	4.1 ± 2.5	5.7 ± 0.5
<b>ESR (mm/h) †</b>	37 (5-60)	22 (13-63)
<b>CRP (mg/l) †</b>	28 (9-52)	62 (3-67)
<b>RF positive (%)</b>	0 (0)	3 (100)
<b>ACPA positive (%)</b>	0 (0)	2 (100)
<b>SJC28 †</b>	2 (2-11)	9 (4-11)
<b>TJC28 †</b>	3 (0-16)	9 (4-11)
<b>VAS †</b>	10 (3-50)	40 (32-73)
<b>US GS †</b>	2 (1-2)	-
<b>US PD †</b>	0 (0-2)	-
<b>NSAIDs (%)</b>	3 (100)	2 (67)

†median (interquartile range); ††mean ± SD; ACPA = anti citrullinated protein antibody; CRP= C-reactive protein; ESR = erythrocyte sedimentation rate; NSAIDs = nonsteroidal anti-inflammatory drugs; RA = rheumatoid arthritis; RF = rheumatoid factor; SJC28 = 28 swollen joint count; TJC28 = 28 tender joint count; US GS = ultrasound greyscale grade at the biopsied joint; US PD = ultrasound power Doppler grade at

the biopsied joint; VAS = global patient visual analogue scale; - = data not obtained from patients at time of presentation

**Table 2. Demographic, clinical and laboratory characteristics of patients in each outcome group.**

	<b>UC (n=2)</b>	<b>CD (n=2)</b>	<b>IBS (n=2)</b>	<b>Metastatic colorectal cancer (n=1)</b>
<b>Age (years)<sup>†</sup></b>	31 (26-36)	36.50 (33-40)	22 (19-25)	44
<b>Female; number (%)</b>	0 (0)	1 (50)	1 (50)	1 (100)
<b>Ethnicity Asian; number (%)</b>	1 (50)	2 (100)	0 (0)	1 (100)
<b>Symptom duration (months)<sup>†</sup></b>	2 (1-3)	5 (3-7)	24 (12-36)	2
<b>Weight (kg)<sup>†</sup></b>	92.70 (76.70-108.70)	85.75 (66.50-105)	67.05 (59.80-74.30)	95.70
<b>Height (m)<sup>†</sup></b>	1.77 (1.75-1.80)	1.68 (1.58-1.79)	1.73 (1.67-1.80)	1.65
<b>BMI<sup>†</sup></b>	29.30 (25-33.60)	29.70 (26.60-32.80)	22.15 (21.40-22.90)	35.20
<b>Smoker; number (%)</b>	0 (0)	0 (0)	1 (50)	1 (100)
<b>FCAL at baseline (µg/g)<sup>††</sup></b>	2302 ± 0	750 ± 963.1	44.50 ± 34.65	583 ± 0
<b>CRP (mg/l)<sup>†</sup></b>	13.5 (3-24)	2.50 (1-4)	2 (1-3)	7
<b>HBI<sup>†</sup></b>	Na	10 (9-11)	na	na
<b>Partial Mayo<sup>†</sup></b>	5 (4-6)	na	na	na
<b>Endoscopic Mayo<sup>†</sup></b>	2 (2-2)	na	na	na
<b>SES-CD<sup>†</sup></b>	Na	3 (3-3)	na	na
<b>Montreal Classification</b>	A2E3S2	A2L1B1	na	na

<b>Treatment</b>	Mesalazine	Budesonide	-	-
<b>(initiated after sampling)</b>	Mesalazine Budesonide Azathioprine			

†median (interquartile range); ††mean ± SD; BMI = Body Mass Index; CD = Crohn's disease; CRP= C-reactive protein; FCAL = fecal calprotectin; HBI = Harvey-Bradshaw Index; IBS = irritable bowel syndrome; na =not applicable; SES-CD = Simple Endoscopic Score for Crohn's disease; UC = Ulcerative colitis, - = data not obtained from patients at time of presentation.

Categorizing race and ethnicity= White and Asian.

**Table 3. Demographic, clinical and laboratory characteristics of IBD patients.**