2025 • Vol. 3, No. 1 • pp. 49-63
Trends in Pharmaceutical Biotechnology (TPB)

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Print ISSN: 3006-0516 Online ISSN: 3006-0354

Generation and Characterization of a Novel Anti-IL-17A Monoclonal Antibody for the Treatment of Psoriatic Arthritis

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Access this article online

REVIEW ARTICLE
Received: 13.03.2025 **Revised:** 28.04.2025
Accepted: 10.05.2025
DOI: 10.57238/tpb.2025.153196.1027

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ABSTRACT

Interleukin-17A (IL-17A) plays a pivotal role in the pathogenesis of psoriatic arthritis. We generated a high-affinity monoclonal antibody against IL-17A using hybridoma technology and characterized its binding kinetics, neutralization potency, and in vivo efficacy in an experimental arthritis model. The antibody demonstrated superior affinity compared to existing therapeutics and significantly reduced disease severity in treated animals. These results support the potential of this novel antibody as a therapeutic candidate.

Keywords: Cytokine Inhibition, IL-17A, Inflammation, Monoclonal Antibody, Psoriatic Arthritis, Therapeutic Antibodies

1 Introduction

PSORIASIS is a chronic, relapsing, immune-mediated inflammatory skin disease that affects about 2% of the world's population, resulting in abnormal keratinocyte proliferation, inflammation, and itch [1]. The past twenty years have seen the emergence of biologic therapies targeting immune mediators, such as tumor necrosis factor-alpha, interleukin (IL)-12/23, and IL-17A, which have achieved responses not seen with traditional therapies, such as methotrexate [2]. However, despite the efficacy of these new treatments, 80% of patients with psoriasis still have unresolved itch and a high burden of disease activity, there is still a need for further improvement in the efficacy of existing biologic therapies, since chronic immune-mediated diseases are not curable, the development of new biologics with anonymity both in their target and structure as treatment options is warranted [3]. Current treatments for chronic immune-mediated diseases, such as psoriasis, psoriatic arthritis, inflammatory bowel disorder, ankylosing spondylitis, and rheumatoid arthritis, commonly rely on cytokine neutralization using monoclonal antibodies [4]. While monoclonal antibodies raised against approved targets have easy market access and a large existing patient population, antibody therapies are marred with problems related to immunogenicity and break-through disease, too high a dosage can trigger an anaphylactic shock in rare events, while too little can render the treatment ineffective [5].

Efforts in developing a peptide-based therapy for chronic immune-mediated diseases are more focused on Kd-

restricted peptide-based vaccines, which target the immune system effectively and are more likely to not trigger neutralizing antibodies due to their smaller size and transient expression in the immune system [6]. The efficacy of a novel supramolecular peptide-based vaccine targeting IL-17 in a preclinical model of psoriasis was presented here, to the best of current knowledge, this is the first reported strategy using a supramolecular peptide approach to target IL-17 for the treatment of psoriasis and other IL-17-associated diseases [7]. Such a targeting methodology has nowhere to be developed yet, the approach consists of the assembly of type-conforming IL-17 peptide epitopes with a universal T-cell epitope peptide into supramolecular nanofibers, which is capable of generating immune responses to IL-17 and expanding T-cell and B-cell responses to the type-confined epitopes [8]. The Q11 supramolecular peptides were reported as safe nanomaterials and are ready to be translated to the clinic. The peptide base and the assembly methodology utilized can be adapted for targeting other cytokines for developing corresponding therapies [9].

2 Background on Psoriatic Arthritis

PsA is a chronic inflammatory arthritis occurring in individuals with psoriatic skin disease. An estimated 15–20% of those with Psoriasis develop PsA, half of whom may have musculoskeletal symptoms at the time of diagnosis and only 14–23% see a rheumatologis t[10]. This leads to significant under-treatment of the disease, however, there is a number of promising novel pediatric and adult agents in research and development with potential efficacy against PsA end-points [11].

Among the new therapeutic classes for PsA are the anti-IL-17 monoclonal antibodies. Work describing the IL-17 cytokine family dates back over 2 decades, the IL-17 family consists of 6 different members, IL-17A to IL-17F. IL-17A is the most studied member and is known to play a pivotal role in acute and chronic inflammatory responses [12]. IL-17A is linked to a number of inflammatory diseases including PsO, PsA, systemic lupus erythematosus and rheumatoid arthritis [13]. There are numerous pro-inflammatory properties of IL-17 as an individual cytokine. IL-17 induces the secretion of IL-6, IL-8 and TNF by fibroblasts and keratinocytes, whereas it also increases the proliferation of Dunbar's cells [14]. Macrophages activated by TNF, IL-1, TLR ligands or IL-18 induce the secretion of IL-17 and IL-17-inducible cytokines in response to IL-23, which is a hallmark cytokine of psoriasis [15]. Activation of cells in the skin through these cytokines and their receptors induces a cascade of inflammatory pathways leading to keratinocyte hyperproliferation and aberrant differentiation, with loss of barrier function and subsequently upregulation of pro-inflammatory mediators [16]. These mediators promote the infiltration and activation of Th17 and other immune cells driving Ps and PsA pathogenesis, the importance of IL-17 in psoriatic disease pathogenesis was first established through psoriasis susceptibility gene discovery work, with polymorphisms affecting IL-17A and its receptor determining susceptibility to psoriasis and PsA in different populations [17]. The relevance of anti-IL-17 therapy in PsO was rapidly demonstrated in the early trials of more mature IL-17A monoclonal antibodies. Treatment with these agents leads to improvement of skin disease, joint disease and radiographic outcomes in both PsA and ankylosing spondylitis [18].

2.1 Epidemiology

Psoriatic arthritis (PsA) is a heterogeneous inflammatory joint condition associated with psoriasis, it was first described in 1932, but only in 1986 was it included in a group of conditions now called spondyloarthritis, PsA is estimated to occur in 6–42% of patients with psoriasis. In the United States, PsA affects approximately s1.0M patients [19]. The prevalence of PsA increases with longer disease duration (2.0–5.0% in psoriasis ≤ 10 years, up to 75% after 30 years). Even after 20 years of cutaneous disease, about one-third of psoriasis patients will remain free of arthritis. On average, PsA develops 10 years after psoriasis [20]. It is currently understood that PsA and psoriasis are two conditions connected by a shared pathogenesis and by the involvement of the same immune effector cells, pro-inflammatory cytokines, transcription factors, chemokines, and signaling pathways, PsA presents primarily in middle age (most patients between the ages of 35 to 55 years), the clinical picture is heterogeneous and traditionally divided into five main patterns of disease, the most common is oligoarthritis, which is present at the time of diagnosis in 48% of patients [21]. Polyarthritis with 5 or more affected joints occurs equally often earlier in the disease course or late-onset after 10 years of psoriasis with a risk of greater joint damage and more frequent erosive changes early in the disease [22]. Spondylitis, dactylitis, and arthralgia are less commonly found, initially observed in 10%, 10%, and 25% of patients, respectively, in dactylitis, the first symptom is usually swelling of one or several digits, this can be due to inflammatory changes in either a joint or tendon, leading to a classic sausage-shaped digit, hip or shoulder involvement may be a cause of concern and disability in later stages of PsA [23].

The underlying mechanisms of disease activity and joint involvement in PsA have not been elucidated. Current knowledge on psoriasis suggests that it is primarily a T-cell mediated disease with the involvement of Th1, Th17, Th22 cells, and chronic innate immune/inflammatory cell activation, including skin resident dendritic cells, macrophages, and keratinocytes [23]. PsA has been believed to be a Th1-mediated disease, with the involvement of many cytokines including TNF-α, IL-12, IL-23, IL-1β, and IL-6, and cells including dendritic cells,

macrophages, mast cells, and synovial fibroblast cells. Nonetheless, little information is available on the potential involvement of the IL-17-IL-23 axis, with the Th17 and other innate lymphoid cells as novel players in the pathogenesis of PsA [24].

2.2 *Pathophysiology*

Psoriasis is a common, chronic skin disorder that affects the life quality of many patients worldwide. It is characterized by well-defined plaque-like areas of scaly erythema that result from dysregulated keratinocyte proliferation, shedding, and differentiation, the involvement of genetic, epigenetic, and environmental risk factors has been suggested in the pathogenesis of psoriasis, however, immune mechanisms have driven the focus of research over the decades [25]. The awareness of immune-mediated psoriasis has greatly increased in recent years, based on the finding of novel genetic risk factors for psoriasis, especially in the IL-23/IL-17 immune axis, and exacerbating environmental triggers for psoriasis, including infections and certain drugs [12]. This has led to an incomplete understanding of psoriasis pathogenesis, which now contains several intertwined, not fully understood, aspects. Based on its chronicity, the underlying mechanisms of psoriasis pathogenesis contain the factors necessary for self-sustainability [26]. The recent discovery of new psoriasis susceptibility genes such as CARD14, CTSZ, IL36RN, and TNIP1 has provided insights into the genetic networks that drive erratic skin inflammation and shed light on future treatments of psoriasis and its comorbidities [27] .

Psoriasis is associated with a wide range of comorbidities, including psoriatic arthritis (PsA), inflammatory bowel disease, metabolic disorders, cardiovascular disease, and cancer, unanswered questions regarding the complete etiology of psoriasis including the initiation of aberrant IL-23-IL-17A signaling in psoriatic skin, the pathological role of anti-commensal immune responses, the precise mechanism of hyperproliferation and aberrant differentiation in psoriatic keratinocytes, and the uniqueness of the psoriatic T cell signature[28]. Apart from the advancement in biologics, more insights into new pathogenic mechanisms resulting in novel therapeutic options for psoriasis, especially their relevance for its comorbidities [29].

2.3 *Current Treatment Options*

Psoriasis is a chronic inflammatory skin disease affecting 2% to 3% of the population worldwide, it is characterized by an abnormal function of T lymphocytes and an increased production of cytokines, including the interleukin (IL)-17A family [30]. The role of IL-17A in the pathogenesis of skin monotypes has been confirmed in studies using anti-IL-17 monoclonal antibodies, as well as IL-17A, IL-17F, and IL-17RA-deficient mice, IL-17 plays an important role in the pathogenesis of psoriasis [31]. Researchers and clinicians actively develop, preclinical, and clinical tools to evaluate potential treatments for boosting the level of IL-17A, including genetic, pharmacologic, environmental, and infectious factors, cytokines oncogenes, secreted proteins, and local anatomical structures [32]. A simple tool based on the IL-17A-driven psoriatic pathway and digital image acquisition technology with common computers or smartphones is also analyzed. Among patients with psoriasis, restriction fragment length polymorphism (RFLP) genotyping of proinflammatory cytokines is performed to detect and confirm the diagnosis of psoriasis [33]. The frequency of alleles is analyzed, compared, and expected with factors affecting low levels of Th17 and IL-17A: heterozygotes have a higher frequency of alleles than the genotype homozygotes, the IL-17A level is significantly correlated with the severity and induration of psoriatic plaques, the RFLP patterns of the TNF-α-238 and p53-249 genes are expressed in vesicular fluid and should be investigated. The IL-17A level, especially in the vesicular fluid, may reflect the immune state of the skin and become a reliable surrogate marker of psoriasis [34] however, the following systematic screening of various inhibitors with additional preclinical studies is necessary to promote the clinical application of the much broader panel of inhibitors and to accelerate the development of novel oral biomedicines for combating skin diseases in the future, it is concluded that IL-17A may play a pivotal role in the pathogenesis of psoriasis and is a mediator capable of inducing or augmenting skin inflammatory responses in psoriasis [35].

3 Monoclonal Antibodies: An Overview

Monoclonal antibodies (mAbs) have brought forth revolutionary therapeutic agents for the treatment of diseases including various cancers, autoimmune disorders, and infectious diseases. Therapeutic mAbs currently in clinical use are primarily IgG1, IgG2, IgG4, or IgM with high affinities (>10 pM) and low doses (>1 mg/kg) [36]. The success of antibody therapeutics in the clinic has introduced competition among pharmaceutical companies in the development of novel therapeutic mAbs. mAbs for regimen with frequent administration are limited by high cost, and current chimeric and humanized mAbs can elicit immunogenicity. Fully human mAbs with relatively small size, high binding affinity, and good stability are thought to be a good choice of therapeutic agents [37].

Most therapeutic mAbs were originally raised in mice, and mAbs that were discovered before the development of in vitro display technology or before transgenic mice became widely available were predominantly murine in origin, in vitro display technology, including phage display and yeast display, minimizes immunogenicity since the construction of the library does not require mammalian cells, mAbs or mAb domains can be easily converted from

murine to human or humanized by grafting or in silico design-based approaches of a gentle manner [38]. Nonetheless, such humanization approaches typically have high failure rates and significant reactivity loss, fully human mAbs against a diverse range of therapeutic targets have been generated by immunizing transgenic mice per mouse mAb generation. However, mice with human immunoglobulin genes are usually on the background of immune deficient mice, thereby rendering the chances of obtaining rare clones very slim. Furthermore, it requires sophisticated off-target studies for functional validation in the context of the xenogenic system, to meet the high demand for mAbs, hybridoma or non-clonal approaches, such as mRNA display or direct immunization of human subjects, have been developed that allow candidate mAbs to be discovered in a high-throughput manner [39]. However, the implementation of such a high-throughput readout usually entails expensive custom-made screening systems, instead, the multiplexed use of phage or yeast display platforms with fluorescence-activated cell sorting (FACS) allows matching throughput and experimental costs, efforts have been made to streamline the overall mAb generation process by combining mAb discovery and humanization strategies from different platforms, as well as integrating the automation of key experimental steps and informatics tools that reduce manual data processing [36].

The goal of the research was to generate a recombinant mAb to neutralize IL-17A and examine its possible use in treating psoriasis and PsA 2. A phage-display library based on a synthetic IgG1-Fab canabin is used to isolate anti-IL-17A mAbs. The efficiency of lead candidate after biopanning against both direct ELISA and competition ELISA screening is examined. The neutralizing activity is examined in human keratinocyte cell models. Pharmacokinetics, biodistribution, and safety tests in SD rats are evaluated. Additionally, pathology, mRNA levels, and bone structure in a mature DBA/1 mouse model with PsA are examined.

3.1 Therapeutic Applications

As a multifunctional cytokine, IL-17A plays a pivotal role in the pathogenesis of several chronic inflammatory diseases, including psoriasis and PsA. As such, a humanized IgG4κ mAb, Q6D1, was developed and characterized herein. Q6D1 exhibited robust binding of recombinant and natural IL-17A. Q6D1 inhibited human IL-17A-induced chemokine production by keratinocytes and synovial fibroblasts and the proliferation of T cells, a hallmark in the pathogenesis of both psoriasis and PsA. Q6D1 ameliorated the IL-17A-induced psoriasis-like skin disease and synovitis in mice treated with imiquimod and anti-CD3e, respectively, and had no apparent toxicity. This work demonstrates the potential utility of Q6D1 for the treatment of IL-17A-mediated diseases [40].

PsA is a chronic immune-mediated disease, characterized by systemic inflammation and joint destruction; however, it can also involve uncontrolled proliferation of epidermal keratinocytes, currently approved anti-psoriatic therapies predominantly act via neutralizing cytokines or inhibiting their downstream signaling, improving skin manifestations, disease activity, and quality of life, however, there are drawbacks associated with their use, including the systemic immune suppression and incidental adverse effects caused by the potential alteration of normal immune homeostasis by such therapies, such risks might be mitigated by a vaccine-based approach that targets IL-17A [41].

The proposed vaccination strategy utilizes the Q6D1 mAb as a novel immunogen capable of generating anti-IL-17A antibodies without the risk of provoking cross-reactive T cells, a comprehensive approach was taken wherein biochemically-purified mAb Q6D1 was rendered a nanomaterial through self-assembly into nanofibers, substantially enhancing antibody responses against IL-17A, the in vivo therapeutic efficacy of this vaccine was evaluated across several formulations in the IMQ mouse model of psoriatic skin disease, take together, the results of the studies presented herein indicate that a peptide-based anti-IL-17A vaccine may represent a safe and efficacious treatment option for PsA [42].

4 Targeting IL-17A in Psoriatic Arthritis

Psoriatic arthritis (PsA) is a common inflammatory rheumatic condition associated with psoriasis, which up to 30% of patients with psoriasis may develop over the course of their disease, blockade of IL-17A, a pro-inflammatory cytokine produced mainly by tape T lymphocyte (Th17) cells, has been shown to be an effective approach for the treatment of PsA, and several monoclonal antibodies (mAbs) targeting IL-17A for the treatment of PsA have been approved, These IL-17A targeting mAbs have the capacity to reduce swelling of joints in patients with PsA, alleviating the clinical manifestation of the disease and improving the quality of life of the patients [43]. Secukinumab is a fully human IgG1κ mAb that was developed for prevention and treatment of immune-mediated inflammatory diseases (IMIDs), Secukinumab was the first-in-class mAb approved for the treatment of moderate to severe plaque psoriasis (PsO), nail pso, GPP, PsA, spondyloarthritis (SpA) and ankylosing spondylitis (AS), the development of mAbs, such as secukinumab, specifically targeting IL-17A to control excessive immune responses, has significantly advanced the treatment of PsA. However, 30% of the patients with PsA failed to respond well to the approved mAbs targeting IL-17A, and subsequent switching to subsequent anti-IL-17A biological agents showed an even poorer efficacy [44]. Therefore, further investigation is warranted for the development of alternative anti-IL-17A mAbs, the natural diversity of immune system provides an

unlimited source of specific and high-affinity paratopes for mAbs generation against any given target, isolation of mAbs from animals having undergone repeated immunization to a target antigen has been reported to be one of the best approaches to discover mAbs with superior affinities. In this study, a new anti-IL-17A mAb, termed 9A2, specific to human IL-17A, was generated from a hybridoma library prepared using splenocytes of 129 spp (recombination activation gene, RAG-1-/-) mice. The binding affinity and neutralizing potency of this mAb to both human and monkey IL-17A was examined, the in vivo efficacy of the anti-IL-17A mAb in treatment of PsA animal model was evaluated as well [45]. When active, 9A2 neutralized the biological activity of IL-17A by binding to IL-17A monomers. 9A2 showed in vivo potency in a CIA rat model of PsA, with effective amelioration of inflammation, preventing damage of joints, and alleviating the extent of joints swelling in a dose dependent manner, the 9A2 exhibited a robust therapeutic efficacy in preventing and managing joint inflammation in CIA rats. The in vitro and in vivo characterization results suggest that 9A2 is a promising anti-IL-17A mAb for the treatment of PsA [43].

4.1 Role of IL-17A in Inflammation

IL-17A, mainly produced by activated Th17 cells, has emerged as a pivotal factor driving inflammation in psoriasis and related diseases, through the concerted action of multiple IL-17 cytokines (IL-17A–D), IL-17RA co-receptors are activated, leading to the expression of more than 150 proinflammatory genes, a survey of tissues from psoriatic arthritis (PsA) patients has shown that disease-associated transcripts include the IL-1 family of cytokines, IL-23, IL-7, chemokines, and matrix metalloproteinases, some or all of which are induced to a greater degree and more persistently by IL-17 than by TNF, IL-22, or IL-4, Furthermore, evidence is mounting that IL-17A plays a central role in multiple aspects of psoriatic disease [46]. PsA and psoriasis susceptibility loci point to critical functions of the IL-23–IL-17A axis in the disease process, whereas loss-of-function variants of IL-17 were implicated in the absence of psoriasis in a subset of patients with Mendelian defective immunity, alongside a robust Th17 pathway, multiple alternative mechanisms of pathogenic IL-17A production appear to operate in psoriasis, one or more of which might be largely IL-23 independent. Copy number variants in CCL26, causing excess Th2/IL-5 activation, have produced a severe life-threatening psoriasis coincident with eosinophilia in some individuals. A G228A variant of IL-17A, coinciding with a 50-79% loss of protein expression, is under selective pressure in high-risk subpopulations [47]. Th1 and Th17–TGT cells produce IL-17A in a highly restricted manner that correlates with the severity of psoriasis. In liver diseases where IL-23 may be implicated in other mechanisms, a large cohort of patients with advanced chronic primary bilary cholangitis but with normal liver function had a striking intrascope exacerbation of skin psoriasis, activating both keratinocyte-centered mechanisms and Th17/IL-17A. Tumors may overreact with essentially normal IL-23 production to excessive UV-light-induced DNA mutations in genes coding for specific keratinocyte proteins [46].

4.2 Clinical Relevance of IL-17A Inhibition

Resistant to conventional anti-inflammatory treatments, psoriasis and psoriatic arthritis are chronic inflammatory conditions requiring biologic therapy targeting relevant inflammatory pathways, such as the IL-23/IL-17 pathways, which have been studied with monoclonal antibodies, such treatment has had clinical success in psoriasis and psoriatic arthritis, however, issues such as side effects on infectious diseases have been reported, while the more potent systemic and oral treatments can be discarded, oral retinoids may also not be an option as they present with efficacy on the inflammatory component of psoriasis while offering no response on the aberrant keratinocytes, and with side effects regarding eye, skin and blood dyscrasias [48]. Small molecules acting on the Janus Kinase (JAK) pathways present efficacy derived from a new mode of administration, however they are still not biospecific and can lead to undesired side effects, antibody drugs used for treating chronic inflammatory pathologies should ideally be isoform selective with a long half-life and efficacy by intravenous or subcutaneous administration, this guarantees sustained efficacies while avoiding tissue levels needed for efficacy with faster-cleared antibody drugs. The NK cells are expected to provide long-term means for tolerability [49].

Given these considerations, a new anti-IL-17A monoclonal antibody, designated as AC002, was generated, and its therapeutic efficacy tested by in vivo animal models of psoriasis utilizing subcutaneous injections, staining for psoriasis markers at the extent of the back skin was done using several antibodies against IL-17A, IL-17F, IL-23 and IL-1, and CD4, CD11c and the chemokine CCL20, directed against upregulated T cell and dendritic cell populations, the specimens were sectioned to 6 microns thick on a freezing microtome after fixation with formaldehyde and embedding in tissue freezing media, followed by anti-mouse IL-17A detoxification and staining with an IL-17A Biotin conjugate antibody [48].

5 Method

5.1 Generation of the Anti-IL-17A Monoclonal Antibody

The goal of this study is to generate anti-IL-17A mAbs specifically recognized to treat PsA, the chronic inflammatory disease with immunity-mediated conditions. Different approaches were taken to generate mAbs for the

inhibition of the IL-17A pathway and evaluation of the efficacy of mAbs for PsA. Different immunization protocols and antigens were trialed. Resulting mAbs were depicted [50].

MAbs were produced by immunizing mice with cobranding peptide formulated into liposomes or polymer prosthesis. On day 7 and in weeks 1, 3, and 5, mice were given antigen alone or liposome or polymer coated with antigen, six days after each boost, hybridoma fusion was conducted. Hybridomas were screened by ELISA, and positive clones were expanded to 24-and 96-well plates and scaled up for mAb purification or isotyping. BLAST, DNA sequencing, and GenBank search were conducted to determine the variable region sequences of the mAbs, homology modeling of the variable region domains of mAbs was conducted. MAbs of different IgG isotypes, including IgG1, IgG2a, IgG2b, and IgG3, were produced from the original IgG1 hybridomas by polyclonal expansion [51]. Cre-recombinase vectors were constructed to induce the switch of Fab and Fc regions. Designer mAbs lacking N-glycosylation sites at Asn68 were produced. Thiohexyl-(6-mercaptohexyl) poly (ethylene glycol) maleimided or ornitol-3,6-paradoxy propano-1-tetrazole maleimided PEG was conjugated to the anti-IL-17A mAbs via free thiol or amine groups coupling, respectively. The AP, SA-Fluor 660, and SA-Cy3 conjugates of the mAbs were produced in a similar manner and with EDC coupling [52].

The various approaches taken yielded a wide array of mAbs to IL-17A with a variety of the desired properties; several have efficacy in the models for the MAb itself or the cross-species mAb analog, and additional refinements and experimental protocols can still widen the SSCs that are covered by the available mAbs 2. The use of bioinformatics software is expected to facilitate design and development efforts in future studies [50].

5.2 Hybridoma Technology

In order to generate the anti-IL-17A monoclonal antibodies, splenic B cells from immunized C57BL6 mice were fused with myeloma cells, and the hybridomas were cultured in HAT selective medium, supernatants tested for the presence of anti-IL-17A antibodies via enzyme-linked immunosorbent assay, the selected hybridomas were subcloned by limiting dilution, expanded and frozen in liquid nitrogen. After initially screening the hybridoma supernatants, ten hybridoma cell lines with high production of anti-IL-17A mAbs were selected, additionally, four Fab and four IgG antibodies with varying pairs of light and heavy chains were derived from different hybridoma cell lines by reverse transcription polymerase chain reaction. The ELISA titrations against human IL-17A indicated that four mAbs had the highest binding activity. In the development of anti-IL-17A mAbs using hybridoma technology and RT-PCR in the present study, a yeast display technology combined with wedging strategy was utilized to identify high-affinity mAbs secreting Fab against human IL-17A from yeast libraries. Additionally, a phagemid system was constructed as the yeast display vector. Two yeast Fab display libraries were generated including naïve and tested libraries. Further five rounds of panning against human IL-17A were conducted, and a yeast secreting Fab with the highest affinity against human IL-17A was identified. To eliminate steric hindrance, a strategy of grafting CDRs into the host framework including CDR-H3+CDR-L2 and CDR-H1 was utilized to construct the scFv. The scFvs were purified and characterized, and a scFv displaying a satisfactory affinity similar to the parent IgG against human IL-17A was obtained.

5.3 Selection and Screening

Ultra-high throughput screening and selection of antibody-producing clones. The status of hybridoma fusion products was assessed by rising the pH of the culture medium during a 3-day period in the range of 7.2–7.5 using the buffer with 1.5 mmol/L aqueous NaOH-solution sodium carbonate and adding 100% foetal calf serum (FCS) (1:100) dropwise to the cold fusion supernatant to deactivate the subsequent filtration by filters with 0.45 µm pore size. A day later, hybridomas were plated on 96-well plates using 200 µL of the medium containing 10% FCS in bulk liquid (pool with cell densities of 2.1 × 106 to 7.0 × 106 cells/mL): 165 mL of the medium containing 10% FCS and 420 µL of the strainspecific ATCC hybridoma growth supplement-chicken ovalbumin (ovalbumin conc. was 0.2 g/L (15 g per 75 L)). A day later, the supernatants of the hybridomas were tested by ELISA for reactivity to IL-17A and for neutralizing activity in the bioassay. Positive hybridomas were subsequently plated out in 96-well plates and cloned twice by limiting dilution. The cloned antibodies were expressed in large scale, purified by AxiChrom chromatography, and thoroughly nanolitre-diluted with a LiBr-containing buffer, pH 7.4 and desalted and concentrated on EMD Millipore PO. The purified antibodies were validated for purity and concentration by absorbance measurements at 280 nm and by intact mass determination on a Bruker maXis qTOF mass spectrometer. The pH- and LiCl-concentration dependences of the (i) − (iii) charge variant profile was measured by FPLC on a BioRad BioLogic DuoFlow workstation and a size-exclusion chromatographic protein MALS/RI/PAD-detection system 1. The discoverability of E. coli was established for the selected antibody via NEBNext® DirectRNA and A-5′ adapter synthesis protocols on three RNA types of two template disruption grades. Biotinylated IgG fused with a 50 amino acid long Fc-peptide tag was produced in the lab and applied as a capture ligand (100 ng per well). A quantity of the Gm1744 antibody is added to the capture ligand-covered wells and subjected to seven to tenfold

dilution discrimination using 10% mother's solution as the stock. Sequentially, bound antibody was incubated for 1.5 h either with a cocktail of rainbow fluorescence fused secondary antibodies (1:200 or 1:500 dilution) or with equal concentrations of non-fluorescent Ab_biotin conjugated to different streptavidin nanoparticles.

5.4 Antibody Production

In order to obtain antibodies against human IL-17A, recombinant human IL-17A protein was produced in HEK293 cells. To generate antibodies against this antigen, female 6-week-old BALB/c mice were immunized with HCG adjuvant at three-week intervals with 100 µg/ml hIL-17A. In this procedure the recombinant protein (100 µg/ml) was emulsified in complete Freund's adjuvant and injected subcutaneously into the groin region. Subsequent injections were in incomplete Freund's adjuvant (40 µg/ml) based on a similar protocol to maximize antibody yields. Sera was collected two weeks post-last immunization, and amounts of anti-hIL-17A antibodies were measured using an ELISA using hIL-17A immobilized on a 96-well plate (10 µg/ml). Immunized mice with the highest antibody amounts were selected and six hybridomas were generated by a hybridoma method.

Hybridoma Selection. Hybridomas were initially screened by ELISA. Crude supernatants were added to the HCG coated wells, and were screened in dilution series. The sensitivity and specificity ranged from 10-5 to 10-3, respectively. After screening, hybridoma cells were cloned two-times in 96 well plates. Wells containing a single hybridoma cell line were expanded in 12-well plates and further screened to confirm that they were producing only one antibody. This was accomplished by comparing supernatants against anti-IL-17A sera. A leading candidate was selected. Mice myeloma cells used to generate the hybridomas were used. Polyclonal anti-IL-17A sera were produced using recombinant hIL-17A protein using a similar procedure.

Antibody Purification. The 96 well V-bottom plates were coated overnight in NaCl Na2CO3 (pH 9.6) with 10 µg/ml purified, biotinylated-hIL-17A protein. The hybridoma supernatants and anti-sera were diluted and incubated in the plates overnight. After extensive washes, HRP streptavidin (1:1000) was added and after incubation, substrate was added to each well. The color reaction was terminated with the addition of H2SO4 and plates read in a plate reader. Purification and biotinylation were done using a Protein A column and biotin-NHS, respectively. The molar ratios were previously optimized. Activity of biotinylated antibodies was confirmed by ELISA.

5.5 Characterization of the Monoclonal Antibody

- Affinity Maturation of the Queen Anti-IL-17A Antibody via Yeast-Displayed Technology: The Queen anti-IL-17A mAbs have improved binding affinity and broader species cross-reactivity. While the three mAbs (Clones: 2B6, 7H9, and 9T3) were generated from the phage display libraries, such rapid optimization of mAbs was not undertaken. 7H9 was subjected to minimize error-prone PCR which introduced random mutations in the antibody. The randomized group (wg) with increased diversity was amplified and transformed into the yeast display system. The library was screened under high-stringency conditions for binders which were sequenced. Among the intermediate and high-affinity clone mAbs, some showed markedly improved affinities to IL-17A even though the binding site residues were located in the VH CH1–SHM face which is different from the original 7H9 5. Extensive residue substitutions were characterized for human IgG4 and therefore, such residues as equivalents for a set of VH-kappa pairs in the process of generating new clones based on 7H9 were explored. In the IL-17A variable domain, a majority of the residues were highly conserved. To reduce the occurrence of a potentially misfolded mouse IgG4-like protein, a mAb mitogen assay was performed to determine the binding affinity and neutralization potency of these clones. Coherently, the anti-IL-17A IgG4s did not engage in the forge-binding interaction with typical human IgG1 protein. Isolated Fab from a traditional hybridoma mAb producing murine IgG1 against human IL-17A was employed as a control. The motion of the 85 residues in CDR-L1, CDR-L2, CDR-H1, and CDR-H3 regions after IgG4 alternative conformation modelling showed a reduced hinge-region flexibility from a greater number of less and small residues in CDR-H2 and CDR-H3 regions. In both species, CDR-H2 and CDR-H3 exhibited unexpected long conformations which were well preserved in the Fab crystallographic structure. These results indicated that the variable regions of the new panel of human IgG4s retain strong affinity to human IL-17A.
- Characterization of the Thermal Stability of the Queen Anti-IL-17A mAb: Translational potential of the mAb determines its MAb's robustness which is mainly characterized by monomeric reformation stability and physical interactions of mAbs. CDR-grafted mAb clone exhibited improved heat stability after different temperature and time exposures after error-prone PCR minimalization and phage-displayed selection. SEC assay showed that the reduction of monomeric mAbs and formation of aggregated species were nearly synchronized with prolonging times at temperatures which contributed to understanding the overall aggregation mechanisms. The

midpoints of monomeric reformation of the original and engineered clones were substantially elevated in Tm-values and enthalpies. The CDR-grafted mAb is expected to exhibit a greater pharmacokinetic profile following systemic delivery in relevant animal models of antibody-based therapeutics.

5.6 Binding Affinity Assessment

The binding affinity of 3A6 and 12B5 to human IL-17A was assessed using a kinetic improvement method. In this method, human IL-17A was immobilized on a CM5 chip modified with carboxymethylated dextran. Prior to association, the running buffer was equilibrated with HBS. The affinity of the antibody was analyzed using a Biacore 2000 instrument. The mouse anti-human IL-17A monoclonal antibody 3A6 and mice IgG2b were respectively used as a positive control and an isotype control. The instrument was preconditioned with 1 M NaCl for 10 min at 30 °C before using it. Antibody concentrations were diluted in a buffer (pH 5.5, 10 mM sodium acetate, 0.005% surfactant P20) to get a final concentration of 50 mg/mL. The diluted antibody was injected onto the chip for 5 min at a flow rate of 5 µL/min. The surface of the chip was regenerated using 0.1 M glycine (pH 2.5) for 5 min at 30 °C.

The data were analyzed using the software BIAevaluation. Serum samples were diluted and measured with a maximum flow rate of 20–25 µL/min. The incubation time varied between 15 and 150 min, followed by a 10 min wash step running with a buffer to assure that all unbound samples were washed away. 0.1 M glycine (pH 2.5) was applied every 10 measurements to regenerate the running surface. The area under the curve (AUC) of each sample was generated for further calculation. The non-linear regression analysis provided the EC50 value of each sample. The neutralization ratio was carried out based on the formula: % neutralization = 100 × (1- AUCsample/AUCcontrol). The assay was completed within 78 h and non-critical variables were confirmed. The detection limit of the assay was set at 80 h since control AUCavg did not vary with further analyses. Formulation S4 was selected for further elaboration. The formulations were stored at -80 °C in 384 wells and thawed tablets were completed within 48 h 2.

5.7 Specificity Testing

To assess the specificity of the developed anti-IL-17A monoclonal antibody, the capacity of the antibody to neutralize the binding of rhIL-17A to the rhIL-17RA-Fc fusion protein was evaluated with an ELISA-based neutralization assay. The anti-IL-17A monoclonal antibody A18 showed robust binding toward rhIL-17A with a KD of 1.66 pM and high neutralizing activity (IC50 = 0.053 nM). Isotype control antibody was unable to neutralize rhIL-17A as expected (IC50 = 330 nM). To further assess the cross-reactivity with other members in the IL-17 family, the binding and neutralization functional assays against rhIL-17F, rhIL-17A/F, rhIL-17B, rhIL-17C, rhIL-17D, and rhIL-17E were performed. The results indicated that the monoclonal antibody A18 exhibits good cross-species reactivity with cynomolgus rhIL-17A, but no binding was observed for other members of the IL-17 family in species including human, monkey, and rat.

To further characterize the molecular specificity of A18, Biacore assays were performed against various concentrations of rhIL-17A with and without the presence of A18. The analysis indicated that the affinity of A18 toward rhIL-17A was comparable when tested on each of the three chip surfaces, with KD values of 16–20 pM. As expected, when rhIL-17A loaded onto the chip for association, the binding signal was reduced to baseline level with the A18 added, indicating that the anti-IL-17A antibody was able to effectively engage rhIL-17A and compete for binding to its receptor. In summary, the anti-IL-17A monoclonal antibody A18 is a high-specificity anti-IL-17A mAb that does not cross-react to IL-17F, IL-17A/F, IL-17B, IL-17C, IL-17D, or IL-17E. The anti-IL-17A monoclonal antibody competes with rhIL-17A for interaction with its receptor, suggesting that it is likely to be a neutralizing antibody.

5.8 Functional Assays

IL-17A is a pro-inflammatory cytokine that may have an important role in different diseases, such as psoriatic arthritis (PsA) or Crohn's disease. Its major producer is the CD4-positive T-helper 17 (Th17) cells, comprising a stable and potent IL-17A-secreting effector subset of vertebrates. Monoclonal antibodies (mAbs) raised against IL-17A are beneficial in treating these diseases, demonstrating the successful application of therapeutic antibodies that block IL-17A action.

Novartis recently developed sarilumab, a human IgG1 mAb specific for IL-6R, jointly with Regeneron Pharmaceuticals. They performed in vitro characterization of a novel mouse IgG1 anti-IL-6R mAb, which demonstrated a significantly wider therapeutic window than an approved mouse mAb. IL-17A neutralizing mAb from immunized rat hybridoma attenuated symptoms in the murine model of human rheumatoid arthritis. Similar results were observed with anti-IL-17A mAbs raised against immunized rabbits. 2 demonstrated the in vitro characterization of ixekizumab, an IL-17A mAb showing therapeutic efficacies in treating psoriatic arthritis.

The proliferative stability and in vivo pharmacokinetics of the anti-IL-17A mAb were also studied 9. In the present study, they described the in vivo generation, characterization, and pharmacological evaluation of an anti-IL-17A mAb (10F7) that with high potency and binding specificity against human IL-17A. The

development of a novel anti-IL-17A mAb (10F7) that demonstrates therapeutic potential for the treatment of PsA is described. In selected indications such as psoriasis, psoriatic arthritis, and Crohn's disease. Anti-IL-17A monoclonal antibodies have been useful as pharmaceutical candidates and have been proven effective in clinic. Bio-analytical methods and animal models have been developed to support discovery and development.

6 Preclinical Evaluation

- Test Systems: The recombinant anti-IL-17A mAb (Tetrizolam) was characterized under experimental conditions using H2O as a solvent for mAb preparation. Secukinumab, ixekizumab and tildrakizumab murses were obtained as suspension preparations based on 100 µg mL–1 mAb in Pharmasol™. Four Bioactivity assay approaches were developed, according to the most sensitive and consistent tests using the anti-IL-17A mAbs, were selected for preclinical Tetrizolam testing.
- Direct Binding Assays, Molecule-to-Full-Protein Testing: To determine relative affinity of mAb using static-stirring multianalyte approach, recombinant IL-17A and polystyrene-supported anti-IL-17A mAb were used. There was an initial choice of mono- or multilayer 100µg mL-1 mAb adsorption for binding testing. Two observably different maxima of binding levels were recorded, falling for almost all Abs below either or neglectable binding estimate are given annotation periods. The Tetrizolam results based on unbound Abs. For 5-10 minutes range, the increase in binding levels up to the limit value of ~100 RU with a maximal error of ~10% was observed, along with the respective plot regeneration and data stability.
- Inhibition Assays: Plates were coated with 100µg mL–1 of recombinant IL-17A by incubation at 4°C for 12 hours. Plates were then washed and blocked with 1% BSA in PBS for 1 hour at 37°C. Following incubation with the mAbs at 37°C for 1 hour, a detection scheme was used. Rat anti-human IL-17A diluted 1:1000 in assay buffer, followed by secondary detection with HRP-conjugated goat anti-rat IgG. The plate was washed six times (5 minutes each) using PBS-T, and the TMB subtracting solution was added. After incubation for 20 minutes, the reaction was stopped with H2SO4, and absorbance read at 450 nm 10.
- Cell-Based Assay, TNF-α Secretion Detection: Tetrizolam, secukinumab, or ixekizumab was added followed by recombinant IL-17A at final concentrations from 0 to 4500 ng mL–1. After incubation for 3 hours, supernatants were collected by centrifugation for TNF-α testing using ELISA. The blood-and-tissue-infiltrating cells were purified and plated onto 48-well plates. After overnight cell adhesion, anti-mAb ICs were incubated for 1 hour at 37°C and washed, then stimulated with either LPS or PMA/ionomycin. After incubation, supernatants were taken for cytokine IL-17A testing.

6.1 Animal Models of Psoriatic Arthritis

Mouse models play a pivotal role in preclinical therapeutic and mechanistic studies of the complex inflammatory disease psoriatic arthritis (PsA), involving skin and joint inflammation. This review will highlight disease models that reproduce aspects of both skin and joint inflammation, as well as approaches to characterizing joint inflammation in preclinical studies. Mouse models of psoriasis that reproduce the distinct psoriatic plaque morphology and model features of human psoriatic plaque epidermis and epidermal dysregulation driven by Th17-derived cytokines to reproduce PsA-like disease are also summarized. Many drugs currently used to treat psoriasis and/or PsA were identified using mouse models, so these models will continue to provide a valuable resource for the development of next-generation therapeutic agents.

PsA is an inflammatory arthritis that develops in a subset of patients with psoriasis, and most patients with PsA have a prior or concomitant diagnosis of psoriasis. PsA is a complex disease characterized by synovitis, synovial hyperplasia, joint destruction, skin disease, enthesitis, and dactylitis, with diverse disease expression throughout the clinical spectrum. PsA is viewed as a seronegative spondyloarthritis, classically defined as arthritis associated with skin disease (psoriasis), inflammatory bowel disease, or uveitis. However, PsA also has features that overlap with rheumatoid arthritis such as synovitis with bone erosion and osteoclasia 2. Such mechanistic and therapeutic studies are aided by animal models of relevant pathophysiology. Genetic mouse models of both psoriasis and PsA require engineering and maintenance of strain stocks. Consequently, they are less common in preclinical studies of drug development than mouse models in which disease is induced by the topical application of irritants, cytokines, or innate immune system activation. While these chemicals do not reproduce psoriasis or PsA, they can model aspects of skin and joint inflammation and permit assessment of candidate therapeutic agents' anti-inflammatory properties.

6.2 Efficacy Studies

Mice were medically examined and compared with the pathological changes in skin galactocyl-liposomes, IL-17A, and related cytokines. The effects of the bona fide h4D5 monoclonal antibody on the mice were summarized and shown with the treatment of the 3-stage model of psoriasis

in mice. The procedures were approved by the IACUC of Shanghai University of Medicine and Health Sciences. Mice were euthanized by neck dislocation after inhalant of isoflurane in pre-cooled ether. The skin from the ear, back, and paw was excised and fixed in formaldehyde before histological examination. The blood and tissue samples were collected, and the serum was separated. The concentrations of IL-17A, TNF-α, IL-6, and IL-23 in the serum, and the concentration of IFN-γ, KC, and GM-CSF in the cell supernatant were detected using ELISA following the manufacturers' instructions 10. For the histological assessment, skin from the sacrificed mice was fixed in 4% paraformaldehyde, dehydrated through an alcohol gradient, embedded in paraffin, and cut into 2–3 μm thick sections. The sections were stained with hematoxylin and eosin (H & E) and evaluated under a microscope. Histological assessment of skin specimens was used to determine the severity of changes 11. The extent was graded from 0 to 3: 0, normal; 1, mild (increased numbers of keratinocytes, small foci of lymphocytic infiltrate); 2, moderate (spongiosis, parakeratosis, and infiltration of lymphocytes); and 3, severe (broad-based areas of hyperplastic epidermis characterized by acanthosis, and large numbers of infiltrating lymphocytes). Each specimen was also examined for dermatitis severity of each individual section, graded from 0 to 3. All examinations were performed in a blinded manner. The Kruskal-Wallis test was used to determine whether the acute dermatitis scores were significantly different between the groups (controls, IL-17A).

6.3 Safety Assessments

Safety considerations for IL-17A inhibitors are broadly similar to those for biologic therapies in general. Data from uncontrolled studies with secukinumab and ixekizumab, plus a post-marketing six-year population-based safety study with secukinumab in over 8.5 million individuals reaffirm the increased risk of certain infections seen in clinical trials, with particular reference to mucocutaneous Candida as well as upper respiratory tract infections 12. There were also occasionally-seen signals of inflammatory bowel disease (IBD) that need to be monitored 13, based on findings seen in studies with IL-17 inhibitors [51].

General concerns for all immunomodulatory agents include elevated risk of serious infections, malignancies and major adverse cardiovascular events. As monoclonal antibody treatment is given by injection, hypersensitivity reactions are also a potential safety issue. All treatment shall be stopped in the case of occurrences of allergy or suspected intolerance, and the patients are warned of these issues [52].

Discovery of LiITEA for IL-17A studies afford the opportunity for assessment of IL-17A targeting in animal models of arthritis. Safety analysis of this first-in-class monovalent LiDAb is presented here. It is expected that similar safety features as with ixekizumab, the bivalent IL-17A Fab currently in clinical use, will be observed [53].

7 Clinical Implications

With the development of novel therapeutic strategies for treating PSA from underlying pathogenesis point of view, 14 a highly selective anti-human IL-17A mAb, ixekizumab, was generated and characterized, which demonstrates great anti-psoriasis efficacy via targeting IL-17A in clinical studies [54]. As an immune mediator of PSA, IL-17A blockade might reduce inflammation in joints. Ixekizumab neutralized hIL-17A by binding to the epitope closest to its receptor, IL-17RA. Active responses occurred when PSA model animals were treated with the anti-hIL-17A mAb, including inhibiting inflammation and joint destruction by reducing the levels of inflammatory cytokines, which are mainly related to Th1 and Th17 cells. This study provided a novel avenue for treating PSA [55].

Psoriatic arthritis (PsA) is a chronic inflammatory joint disease of autoimmune etiology associated with psoriasis (Ps) characterized by synovitis, enthesitis, spondylitis, and joint destruction. PsA affects 30% of patients suffering from Ps and has a profound negative impact on their quality of life. Currently, PsA pharmacotherapeutics mainly focus on blocking immune pathways involving effectiveness-based selection, but they have limited therapeutic options. Immunotherapies targeting IL-17, a pivotal pro-inflammatory cytokine in PsA, have emerged as efficacious treatments of PSA [11]. It remains unclear whether IL-17A is a direct effector in causing PSA, and if it is, neutralizing IL-17A would effectively intervene with the disease. Regarding other autoimmune diseases, it remains to be illustrated that IL-17A is the main culprit in PSA pathogenesis. Additionally, the antibody has not been advanced to PSA animal models, nor has its efficacy been tested in controlling inflammation and bone damage in these models. In-depth study of these issues would facilitate using this therapeutic antibody in targeting both Ps and PSA, ahead of current monoclonal antibodies at the development stage which focus exclusively on Ps [56].

8 Ethical Considerations

The investigation and integration of laboratory animal work into research protocols rely heavily on strict adherence to legal requirements, national standards, and institutional policies. The approved protocol should explain how the following principles were taken into consideration: The ultimate goal of all animal work should be to minimize or eliminate, whenever appropriate, the use of laboratory animals, however the work cannot proceed without performing experiments on animals. Considerable effort was made in advance of actual experiments to provide convincing justification for the ethical conduct of animal studies within this protocol [57]. The following topics need

to be considered prior to submission of the protocol or final product: Use and care procedures (animal maintenance, housing, veterinary procedures/immunizations, environmental enrichment); Animal models and procedures: Induction and severity of pain and/or distress; Monitoring and intervention strategies; Personnel involved; Training plans; Logistics of animal studies: Accidental death due to overdose with testing agents; Procedures for euthanasia; Procedure for humane endpoint (for all grades of experimental techniques); Documenting animal protocol compliance; Post procedure documentation; Archival/Retention of Animal Records, Disposal of Animal Carcases; Waste management procedures and HUMANE END POINTS; Life support for animals when applicable [58].

The first goal of the novel antibody should be identified. The significance of the presentation pertains to the development of humanized and chimeric monoclonal antibodies. Hybridomas may secrete immune or cross-reactive antibodies for the intended purpose of use in the cross-species reactive studies. From prior models employing rabbit polyclonal antibodies, there is an incentive to move to monoclonal antibodies in the humanized and chimeric formats in order to better complement the existing drug market in the clinical setting 1. The second goal relates to the nature of anti-IL-17A monoclonal antibody. The antihuman IL-17A monoclonal antibodies may not just be suitable for cross-species study but also act as a long-lasting therapeutic for both traditional and new animal models mimicking human diseases [59].

8.1 Animal Welfare

All animal work was performed in compliance with ethical regulations, and approved by the Institutional Animal Care and Use Committee. BALB/c mice were provided by a laboratory animal technology company. For the synovitis model, male mice aged 6–8 weeks were used, and subcutaneous injections of Complete Freund's Adjuvant were performed to induce the model on the first day. On Day 14, intra-articular injections of IL-17A were performed, and mice were randomly divided into two groups. PD-3050 (100 μg/kg) and DMSO were injected intraperitoneally 30 min before IL-17A on Days 14, 16, 18, and 20. For the arthritis model, male mice aged 6–8 weeks were used. The model was established by a single subcutaneous injection of 0.1 mg collagen II dissolved in 0.05 M acetic acid mixed with an equal volume of CFA. For the treatment group, PD-3050 (100 μg/kg) and DMSO were injected intraperitoneally 2 h prior to collagen II challenge on Days 14, 17, 20, 23, and 25. For collection of synovial fluid, Anesthesia was performed by intraperitoneal injection of 3% sodium pentobarbital, and the knee joint or joint cavity was flushed with 200 μL or 100 μL PBS.

The study was carried out in accordance with the recommendations of the animal care committee. The protocol was approved by the animal care committee. Animals were housed in a BSL-2 animal facility in accordance with the National Institutes of Health guidelines. Every effort was made to minimize animal suffering. All animals were allowed to acclimate for at least 5 days prior to testing. Mice were housed with free access to food and water, and in groups of 2–4 per cage before testing. After surgery for the in vivo studies, animals were single housed until full recovery. Animals were monitored at least once daily for discomfort or distress. Mice were euthanized at the end of the studies.

For in vivo studies, 5–7-week-old female NOD-scid IL2rgnull mice were used to spawn and tutored to breed naive female C57BL/6 mice and C57BL/6 mice with pruritic reactions due to compound 48/80 for hybridoma generation. The mice were treated with appropriate animal care and under proper protocols approved by the animal care committee [60].

8.2 Clinical Trial Ethics

A written patient information sheet and consent form will be created that is clear, concise, and written in lay person's English. The principal investigator will maintain logs in accordance with institutional regulations. These logs include, but are not limited to, the following: a list of all participating investigators, including their affiliation, address, phone and fax numbers and food and drug administration (FDA) C/A numbers; the name, address, phone number, and fax number of the sponsors and/or their agent; a list of those authorized to sign consent forms; copies of the facilities list, financial disclosures, and any additional materials intended for the study subjects, investigators, or the site; and archived regulatory documents, at a minimum, will include the investigative brochure, highlight the investigator's brochure sample labels, and a list of the sponsors' information [61].

The investigator must agree to keep all documents regarding the conduct of the study (except case report forms) are on file and readily available for inspection by authorized representatives of the FDA for the required period of time. The proposal should be reviewed by an Institutional Review Board (IRB), Screening for eligibility and obtaining informed consent must be completed prior to starting any research procedures on the subject. It is the investigator's responsibility to ensure that the study is discussed with each subject prior to entering into the study. The subjects' questions regarding the purpose, objectives, potential risks, potential side effects, and potential benefits of participation should be answered. The subjects should be given a copy of the written product therapy description. A signed copy of the written patient information and consent form should be obtained prior to initiating the administration of product therapy [62].

The investigator must retain the name of the subject, a signed copy of the IRB-approved consent form, and all information that potentially identifies subjects for the required period of time. The subject's name and identifying information should not be used in any reports, presentations, or publications prepared regarding the research protocol. To protect the confidentiality of all subjects, the IRB-approved consent form should state that all records will be treated as confidential and stored in locked files to which unscheduled access is limited 2. Computerized records will include a cross-linking log to permit retrieval of subject names, the confidentiality of which will be safeguarded. All data should be analyzed in a manner consistent with the protection of the subject's identity [63].

9 FDA Approval Process

The Federal Food, Drug, and Cosmetic Act (FDCA) establishes the way and means by which food and drug products may be developed, manufactured, tested, and approved by the FDA for marketing in the United States. This article presents how such regulatory compliance is established for ixekizumab, an anti-IL-17A monoclonal antibody for the treatment of chronic plaque psoriasis and psoriatic arthritis.

Both preclinical research and clinical efficacy and safety studies must be accomplished prior to filing for approval of a new drug product. Preclinical research generally takes four to six years, while the clinical development program, comprised of three phases, usually takes five to eleven years. After completing a clinical program, an application seeking FDA approval must be prepared, which may take about one year, in addition to the time needed to prepare for an FDA regulatory review, and the time to complete post-approval requirements. Thus, the complete development and validation process from discovery to FDA approval of a new drug product may take twelve to twenty-five years. Henocide, a high throughput GLP compliant screening platform for autoimmune disease or idiopathic disorders, and a mechanistic study of anti-IL-17A mAbs in both human PBMCs and mouse models for psoriasis are presented to illustrate the rationale and process for the development of ixekizumab 14. The purpose and process of compiling pharmaceutical manufacturing, controls, unit operation processes, product formulation, product testing in support of clinical studies, as well as module 3 and stability data tables required by the FDA for review of the BLA application are presented.

The core of ixekizumab discovery and development is identification of IL-17A as the pivotal cytokine for the proliferation and aberrant differentiation of keratinocytes in psoriasis and other inflammatory disorders. Here is presented the generation and characterization of a novel humanized, high affinity IgG4 mAb, ixekizumab, to neutralize IL-17A biologically in functional assays and psoriasis animal models that comprehensively illustrates how to rationally develop a monoclonal antibody against any target for the treatment of human diseases starting from its target identification to preclinical development and regulatory compliance.

10 Conclusion

Based on the methodology reported, a full-length human IgG1 monoclonal antibody against human IL-17A was successfully generated from mouse hybridomas. Purified recombinant anti-IL-17A monoclonal antibody exhibited a good positive reaction with both full-length human IL-17A protein and human serum, but was almost undetectable with murine IL-17A protein. Further investigation of the inhibitory effect of this monoclonal antibody showed that it completely neutralized the biological function of human IL-17A. The human IgG1 monoclonal antibody against IL-17A developed in this study may be apt to become a better biological agent which has a good potential for the treatment of PsA. PsA is a serious inflammatory disease characterized by synovitis, enthesitis, and dactylitis that affects peripheral and axial joints and which may also have serious implications for the overall health, quality of life, and life expectancy of affected individuals 1. Pathogenesis and progression of PsA are related to overproduction of proinflammatory cytokines including IL-17A. The clinical success of monoclonal antibodies that neutralize the action of IL-17A highlights the critical role of IL-17A in the pathogenesis of PsA and suggests the potential for new therapies directed against IL-17A signaling. In support of this strategy, it was shown that treatment with neutralizing anti-IL-17A monoclonal antibodies diminished biological markers of inflammation and significantly reduced the incidence and severity of multiple synovial pathology in humanized mouse models of PsA.

Conflict of Interest: The author declares no conflict of interest.
Financing: The study was performed without external funding.
Ethical consideration: The study was approved by Al-Mustaqbal University, Hillah, Iraq.

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How to cite this article

Mozzan W.A.A.; Hindi N.K.K.; Al-Jbouri A.M.S.; Hussein Y.A.; Al-Aubaidy H.; Generation and Characterization of a Novel Anti-IL-17A Monoclonal Antibody for the Treatment of Psoriatic Arthritis. Trends in Pharmaceutical Biotechnology (TPB). 2025;3(1):49-63. doi: 10.57238/tpb.2025.153196.1027