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In-vitro and *In-vivo* screening methods for targeting HMBG1 in RA: A comprehensive overview

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Abstract

Targeting High Mobility Group Box 1 (HMGB1) in rheumatoid arthritis (RA) holds promise for mitigating inflammation and joint damage. This paper comprehensively overviews *In Vitro* and *In Vivo* screening methods for HMGB1 targeting in RA. *In Vitro*, assays include cell-based assays, ELISA, and Western blotting to assess HMGB1 release, receptor activation, and downstream signalling pathways. *In Vivo*, models such as collagen-induced arthritis (CIA) in mice and adjuvant-induced arthritis (AIA) in rats mimic RA pathogenesis and enable evaluation of HMGB1 inhibitors' efficacy, safety, and pharmacokinetics. Advanced imaging technologies, including PET and MRI, allow non-invasive visualization of HMGB1 expression *In Vivo*. Biomarker analyses complement screening methods by correlating HMGB1 levels with disease activity and treatment response. Integration of these screening methods facilitates the development of HMGB1-targeted therapies with the potential to transform RA management. In this review we proposed certain *In-vitro* and *In-vivo* screening methods for RA.

Keywords: Rheumatoid arthritis; HMGB1; Pro and inflammatory cytokine; Screening methods; *In-vitro* and *In-vivo*

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by inflammation of the joints, leading to pain, swelling, stiffness, and eventual joint damage. High Mobility Group Box 1 (HMGB1) protein has emerged as a key mediator in the pathogenesis of RA, contributing to inflammation, immune dysregulation, and tissue damage [1, 2]. Targeting HMGB1 represents a promising therapeutic strategy for RA management, but effective implementation requires robust screening methods to evaluate the efficacy, safety, and pharmacokinetics of HMGB1-targeted therapies. *In Vitro* and *In Vivo* screening methods play crucial roles in identifying and optimizing HMGB1-targeted therapies for RA [3]. *In Vitro*, assays provide insights into the molecular mechanisms underlying HMGB1 signalling and allow for screening candidate inhibitors in controlled experimental settings. In contrast, *In Vivo* models recapitulate RA pathogenesis and enable the evaluation of HMGB1 inhibitors' efficacy in complex biological systems [4].

1.1. *In Vitro* Screening Methods

In Vitro, screening methods offer a cost-effective and high-throughput approach to evaluating the effects of HMGB1 inhibitors on cellular signalling pathways and immune responses. Cell-based assays utilizing human cell lines, primary cells, or co-culture systems provide valuable insights into HMGB1 release, receptor activation, and downstream signalling events [5]. These assays allow researchers to assess the potency, selectivity, and mechanism of action of HMGB1 inhibitors under controlled experimental conditions. Enzyme-linked immunosorbent assay (ELISA) and Western blotting are commonly used to quantify HMGB1 levels in cell culture supernatants or biological samples. ELISA-based assays enable the specific detection and quantification of extracellular HMGB1, while Western blotting allows for

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the assessment of HMGB1 expression and post-translational modifications [6, 7]. These techniques provide essential information on HMGB1 release kinetics, cellular localization, and protein-protein interactions in response to inflammatory stimuli. Functional assays targeting HMGB1 receptors, such as Receptor for Advanced Glycation End Products (RAGE) and Toll-like receptors (TLRs), assess the biological activity of HMGB1 and the efficacy of HMGB1 inhibitors in blocking receptor activation [8]. Reporter gene assays, calcium mobilization assays and flow cytometry-based assays measure downstream signalling events, cytokine production, and immune cell activation induced by HMGB1-receptor interactions. These assays facilitate the identification of lead compounds with optimal pharmacological properties for further preclinical evaluation.

1.2. *In Vivo* Screening Methods

In Vivo screening methods provide critical insights into the efficacy, safety, and pharmacokinetics of HMGB1-targeted therapies in complex biological systems. Animal models of RA, such as collagen-induced arthritis (CIA) in mice and adjuvant-induced arthritis (AIA) in rats, replicate key features of human RA pathology, including synovial inflammation, cartilage degradation, and bone erosion [9]. These models enable researchers to assess the therapeutic potential of HMGB1 inhibitors *In Vivo* and evaluate their effects on disease progression, joint damage, and systemic inflammation. Preclinical studies using animal models of RA involve the administration of HMGB1 inhibitors via various routes, including oral gavage, intraperitoneal injection, or intra-articular injection, to assess their pharmacokinetic properties and bioavailability [10, 11]. Disease activity and severity are monitored using clinical scoring systems, histological analysis, and imaging modalities such as micro-computed tomography (micro-CT) and magnetic resonance imaging (MRI). Biomarker analyses, including cytokine profiling, autoantibody measurements, and gene expression profiling, provide insights into the mechanisms of action and therapeutic effects of HMGB1 inhibitors *In Vivo*. Advanced imaging technologies, such as positron emission tomography (PET) and bioluminescence imaging (BLI), enable non-invasive visualization and quantification of HMGB1 expression and distribution in living animals. Molecular imaging probes targeting HMGB1 receptors or ligands facilitate the assessment of HMGB1 dynamics and localization in inflamed joints and tissues [12-15]. These imaging modalities provide valuable information on drug distribution, target engagement, and treatment response *In Vivo*, guiding dose optimization and therapeutic decision-making.

1.3. Integration of *In Vitro* and *In Vivo* Screening Methods

Integration of *In Vitro* and *In Vivo* screening methods is essential for advancing HMGB1-targeted therapies from bench to bedside. *In Vitro* assays provide mechanistic insights into HMGB1 signalling pathways and facilitate the identification of lead compounds with favourable pharmacological properties [16]. *In Vivo*, models complement *In Vitro* findings by assessing the efficacy, safety, and translational potential of HMGB1 inhibitors in disease-relevant contexts. Translational research efforts aim to validate preclinical findings in human subjects and bridge the gap between benchtop experiments and clinical applications. Clinical trials evaluating HMGB1-targeted therapies in RA patients utilize biomarker analyses, imaging modalities, and patient-reported outcomes to assess treatment responses and monitor disease activity over time. Biomarker-guided therapy monitoring enables personalized treatment approaches tailored to individual patient characteristics and treatment responses. Collaborative research efforts involving academia, industry, and regulatory agencies facilitate the translation of preclinical discoveries into clinical practice [17-20]. Consortia, research networks, and collaborative initiatives promote data sharing, resource allocation, and knowledge exchange, accelerating the development and implementation of HMGB1-targeted therapies for RA management. By leveraging the strengths of *In Vitro* and *In Vivo* screening methods, researchers can optimize HMGB1 targeting strategies and improve outcomes for individuals living with RA.

2. Understanding rheumatoid arthritis and HMGB1

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by inflammation of the joints, leading to pain, swelling, stiffness, and eventual joint damage [21]. While the precise etiology of RA remains elusive, a complex interplay of genetic, environmental, and immunological factors contributes to disease pathogenesis. High Mobility Group Box 1 (HMGB1) protein has emerged as a key player in RA, exerting diverse effects on inflammatory and immune responses [22].

2.1. Understanding Rheumatoid Arthritis

Rheumatoid arthritis is a systemic autoimmune disease characterized by chronic inflammation of the synovial membrane, leading to the destruction of cartilage, bone erosion, and joint deformity. The hallmark feature of RA is symmetrical joint involvement, typically affecting the small joints of the hands, wrists, and feet [23, 24]. However, RA can also involve large joints and other organ systems, leading to a wide range of clinical manifestations. The pathogenesis of RA involves a complex cascade of events initiated by immune dysregulation, leading to synovial

inflammation and joint destruction. Genetic predisposition, environmental triggers, and dysregulated immune responses contribute to the development of autoantibodies, such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) [25]. These autoantibodies target self-antigens, such as citrullinated proteins, leading to immune complex formation and activation of inflammatory pathways. Inflammation within the synovium triggers the release of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), perpetuating a state of chronic inflammation. Activated immune cells, including T cells, B cells, and macrophages, infiltrate the synovial tissue, perpetuating the inflammatory cascade and promoting tissue destruction [26]. Over time, chronic inflammation leads to cartilage degradation, bone erosion, and joint deformity, resulting in functional impairment and disability.

2.2. Understanding HMGB1

High Mobility Group Box 1 (HMGB1) is a highly conserved nuclear protein that plays diverse roles in inflammation, immunity, and tissue repair. Originally identified as a DNA-binding protein involved in chromatin structure and gene regulation, HMGB1 has since been recognized as a potent pro-inflammatory cytokine and damage-associated molecular pattern (DAMP) molecule [27, 28]. HMGB1 is ubiquitously expressed in various cell types, including immune cells, epithelial cells, and endothelial cells. Under physiological conditions, HMGB1 is predominantly localized within the cell nucleus, where it facilitates DNA replication, repair, and transcriptional regulation [29]. However, in response to cellular stress, inflammation, or tissue injury, HMGB1 can be actively released into the extracellular milieu, where it functions as an inflammatory mediator. Extracellular HMGB1 acts through multiple receptors, including the Receptor for Advanced Glycation End Products (RAGE), Toll-like receptors (TLRs), and the Receptor for HMGB1 (HMGB1) [30]. Engagement of these receptors by HMGB1 activates downstream signalling pathways, leading to the production of pro-inflammatory cytokines, chemokines, and adhesion molecules. HMGB1 also promotes immune cell activation, migration, and survival, further amplifying the inflammatory response.

2.3. Role of HMGB1 in Rheumatoid Arthritis

In rheumatoid arthritis, HMGB1 contributes to disease pathogenesis through its pro-inflammatory and immunomodulatory activities. Synovial fluid and serum levels of HMGB1 are elevated in RA patients compared to healthy individuals, correlating with disease activity, severity, and progression. HMGB1 expression is upregulated in synovial tissue, cartilage, and bone marrow of RA patients, suggesting a role in local inflammation and joint damage [31, 32]. HMGB1 promotes inflammation within the synovium by stimulating the production of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, from resident synovial cells and infiltrating immune cells. HMGB1 also induces the expression of matrix metalloproteinases (MMPs) and other proteolytic enzymes involved in cartilage degradation and bone resorption. Furthermore, HMGB1 facilitates the recruitment and activation of immune cells, including neutrophils, monocytes, and T cells, exacerbating synovial inflammation and tissue destruction [33-35]. In addition to its pro-inflammatory effects, HMGB1 contributes to the perpetuation of autoimmunity in RA by promoting the generation of autoantibodies and the activation of autoreactive immune cells. HMGB1 induces the expression of citrullinated proteins, which serve as targets for ACPAs, leading to the formation of immune complexes and the activation of complement cascades. HMGB1 also promotes the differentiation and activation of antigen-presenting cells, enhancing the presentation of self-antigens to T cells and perpetuating the autoimmune response [36].

2.4. Therapeutic Targeting of HMGB1 in Rheumatoid Arthritis

Given its central role in RA pathogenesis, HMGB1 has emerged as a promising therapeutic target for RA treatment. Several strategies have been explored to modulate HMGB1 activity, including small molecule inhibitors, monoclonal antibodies, and antagonistic peptides. These HMGB1-targeted therapies aim to inhibit HMGB1 release, neutralize extracellular HMGB1, or disrupt HMGB1-receptor interactions, thereby attenuating inflammation, tissue damage, and autoimmunity in RA. Preclinical studies have demonstrated the efficacy of HMGB1-targeted therapies in ameliorating disease symptoms and joint damage in animal models of RA [37, 38]. HMGB1 inhibitors have been shown to reduce synovial inflammation, cartilage destruction, and bone erosion while improving joint function and mobility. Furthermore, HMGB1 blockade synergizes with existing RA treatments, such as DMARDs and biologics, to enhance therapeutic efficacy and reduce treatment resistance. Clinical trials evaluating HMGB1-targeted therapies in RA patients aim to validate their safety, efficacy, and tolerability in human subjects [39, 40]. These trials utilize biomarkers, imaging modalities, and patient-reported outcomes to assess treatment responses and monitor disease activity over time. Early results from clinical trials suggest that HMGB1 inhibitors hold promise as adjunctive therapies for RA patients refractory to conventional treatments or biologic agents.

2.5. Challenges and Future Directions

Despite the therapeutic potential of HMGB1 targeting in RA, several challenges remain to be addressed. One challenge is the pleiotropic nature of HMGB1, as it exerts diverse effects depending on its cellular localization, redox state, and interaction partners [41]. Developing inhibitors that selectively target pathological HMGB1 signalling while preserving its physiological functions represents a significant hurdle. Another challenge is the heterogeneity of RA, as patients exhibit varying disease phenotypes, treatment responses, and comorbidities. Personalized medicine approaches, integrating patient-specific factors such as genetic polymorphisms, biomarker profiles, and disease activity measures, are needed to tailor HMGB1-targeted therapies to individual patient needs [42, 43]. Furthermore, translating preclinical findings into clinical practice requires rigorous clinical trial design, patient recruitment, and regulatory approval. Collaborative research efforts involving academia, industry, and regulatory agencies are essential for advancing HMGB1 targeting in RA treatment and overcoming these challenges.

3. Exploring *In-vitro* screening methods for HMGB1 targeting

High Mobility Group Box 1 (HMGB1) is a highly conserved protein that plays multifaceted roles in various physiological processes, including inflammation, immunity, and tissue repair. In pathological conditions such as rheumatoid arthritis (RA), HMGB1's dysregulated release and signalling contribute significantly to disease progression [44, 45]. Consequently, targeting HMGB1 has emerged as a promising therapeutic approach for managing RA and other inflammatory disorders. *In-vitro* screening methods form the cornerstone of drug discovery and development, providing crucial insights into HMGB1 biology and facilitating the identification of novel inhibitors.

3.1. Cell-Based Assays

Cell-based assays represent indispensable tools for investigating HMGB1-mediated cellular responses and assessing the efficacy of HMGB1-targeted therapies. These assays utilize cultured cells, including immune cells, synoviocytes, and fibroblasts, to model various aspects of inflammation and autoimmunity [46]. They offer a controlled environment for studying HMGB1 signalling pathways, receptor interactions, and downstream effects. Commonly employed cell-based assays include those evaluating cell viability, proliferation, inflammatory cytokine production, and NF-κB activation. Cell viability assays, such as MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assays, provide quantitative measurements of cell metabolic activity following treatment with HMGB1 inhibitors [47-49]. These assays are instrumental in assessing drug toxicity and determining appropriate drug concentrations for subsequent experiments. Cell proliferation assays, including BrdU (5-bromo-2'-deoxyuridine) incorporation assays and Ki-67 staining, enable the evaluation of HMGB1 inhibitors' effects on cell proliferation and growth. They offer valuable insights into the antiproliferative properties of HMGB1-targeted therapies, particularly relevant for mitigating synovial hyperplasia in RA. Inflammatory cytokine assays, such as ELISA and multiplex cytokine assays, quantify the production of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) in response to HMGB1 stimulation or inhibition [50, 51]. These assays elucidate the immunomodulatory effects of HMGB1 inhibitors on cytokine production and inflammation, providing mechanistic insights into their therapeutic potential. NF-κB reporter assays measure NF-κB transcriptional activity following HMGB1 stimulation or inhibition. Luciferase reporter constructs containing NF-κB response elements are transfected into cells, allowing the quantification of luciferase activity as a readout of NF-κB activation [52, 53]. These assays elucidate the molecular mechanisms underlying HMGB1-induced NF-κB signalling and evaluate the efficacy of NF-κB inhibitors in blocking HMGB1-mediated inflammation.

3.2. Enzyme-Linked Immunosorbent Assay (ELISA) and Western Blotting

Enzyme-linked immunosorbent assay (ELISA) and Western blotting are indispensable techniques for quantifying HMGB1 levels and analysing protein expression and post-translational modifications. ELISA-based assays enable the specific detection and quantification of extracellular HMGB1 in cell culture supernatants or biological samples [54]. They provide valuable information on HMGB1 release kinetics, cellular localization, and dynamics in response to inflammatory stimuli or drug treatment. Western blotting allows for the analysis of HMGB1 expression, localization, and protein-protein interactions in cell lysates or tissue homogenates. By probing with specific antibodies, Western blotting enables the characterization of HMGB1 isoforms, modifications (e.g., acetylation, phosphorylation), and interactions with binding partners. These techniques facilitate the elucidation of HMGB1's role in RA pathogenesis and the evaluation of HMGB1-targeted therapies [55, 56].

3.3. Co-Immunoprecipitation (Co-IP) and Protein-Protein Interaction Studies

Co-immunoprecipitation (Co-IP) and protein-protein interaction studies provide valuable insights into HMGB1's interactions with its binding partners, including receptors, cytokines, and intracellular signalling molecules [57]. Co-IP

assays enable the isolation and identification of HMGB1-associated proteins from cell lysates or tissue extracts using specific antibodies against HMGB1 or its interacting partners. Immunoprecipitated protein complexes are then analysed by Western blotting or mass spectrometry to identify interacting proteins and characterize HMGB1 signalling networks [58]. These studies elucidate the molecular mechanisms underlying HMGB1-mediated inflammation and identify potential targets for therapeutic intervention.

3.4. Surface Plasmon Resonance (SPR) and Biomolecular Interaction Analysis

Surface plasmon resonance (SPR) and biomolecular interaction analysis techniques, such as Bia core and Octet, quantify the binding affinity and kinetics of HMGB1 interactions with ligands, receptors, or small molecules. SPR measures changes in the refractive index of a sensor surface upon biomolecular binding, allowing real-time monitoring of binding kinetics, association and dissociation rates, and equilibrium binding constants [59, 60]. These techniques provide insights into the molecular interactions between HMGB1 and its binding partners, facilitating the design and optimization of HMGB1-targeted therapies with high binding affinity and specificity.

3.5. High-Content Screening (HCS) and Automated Imaging

High-content screening (HCS) and automated imaging platforms enable the high-throughput analysis of cellular phenotypes and drug effects using fluorescent or immunofluorescent labelling techniques. HCS combines automated microscopy, image analysis algorithms, and data mining software to quantify cellular parameters, such as morphology, proliferation, and protein expression, in large-scale screening experiments [61]. These platforms facilitate the screening of compound libraries, identification of novel drug targets, and characterization of drug mechanisms in complex cellular systems. In the context of HMGB1 targeting, HCS platforms can be used to assess the effects of HMGB1 inhibitors on cell morphology, cytokine production, and NF-κB activation in RA-relevant cell types.

4. Developing *in-vivo* screening methods for HMGB1 targeting

Developing *In Vivo* Screening Methods for HMGB1 Targeting in Rheumatoid Arthritis Treatment Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by inflammation of the joints, leading to pain, swelling, and eventually joint damage [62]. High Mobility Group Box 1 (HMGB1) protein has emerged as a potential therapeutic target in RA due to its role in mediating inflammatory responses and tissue damage. While significant progress has been made in understanding HMGB1 biology and developing inhibitors targeting its activity, translating these findings into effective therapies requires robust *In Vivo* screening methods.

4.1. Understanding the Complexity of HMGB1 Signaling

HMGB1 is a multifunctional protein involved in various physiological processes, including inflammation, immunity, and tissue repair. In the context of RA, HMGB1 contributes to disease pathogenesis by promoting inflammation, synovial hyperplasia, and cartilage degradation [63]. Its release from immune cells and damaged tissues amplifies inflammatory responses, perpetuating chronic inflammation and joint damage in RA patients. Targeting HMGB1 presents a promising therapeutic strategy for modulating RA pathogenesis and alleviating disease symptoms.

4.2. Challenges in HMGB1 Targeting

Despite the therapeutic potential of HMGB1 inhibition, several challenges hinder the development of effective HMGB1-targeted therapies. One of the primary challenges is the pleiotropic nature of HMGB1, as it exerts diverse effects depending on its cellular localization, redox state, and interaction partners [64]. Developing inhibitors that selectively target pathological HMGB1 signaling while preserving its physiological functions remains a significant hurdle. Additionally, HMGB1 exists in various isoforms and undergoes post-translational modifications, further complicating drug development efforts.

4.3. Importance of *In Vivo* Screening Methods

In Vivo screening methods are essential for evaluating the efficacy, safety, and pharmacokinetic properties of HMGB1-targeted therapies in relevant disease models [65]. Preclinical animal models of RA, such as collagen-induced arthritis (CIA) in mice and adjuvant-induced arthritis (AIA) in rats, provide valuable platforms for assessing the therapeutic potential of HMGB1 inhibitors. *In Vivo* studies allow researchers to investigate the effects of HMGB1 blockade on disease progression, joint inflammation, cartilage destruction, and systemic immune responses.

4.4. Advances in Imaging Technologies

Recent advances in imaging technologies offer new opportunities for *In Vivo* screening of HMGB1 targeting strategies. Molecular imaging techniques, such as positron emission tomography (PET) and magnetic resonance imaging (MRI), enable non-invasive visualization and quantification of HMGB1 expression in RA joints [66]. By tracking HMGB1 levels longitudinally in live animals, researchers can monitor treatment responses and optimize therapeutic regimens in real time. Moreover, molecular imaging allows for the assessment of drug distribution, target engagement, and off-target effects *In Vivo*.

4.5. Integration of Biomarkers

Biomarkers play a crucial role in guiding HMGB1 targeting strategies and predicting treatment responses in RA patients. *In Vivo* screening methods can incorporate biomarker analyses to correlate changes in HMGB1 levels with disease activity, joint damage, and treatment outcomes [67, 68]. Biomarker-based approaches facilitate the identification of patient subpopulations most likely to benefit from HMGB1-targeted therapies, enabling personalized treatment strategies in RA management.

4.6. Validation in Translational Models

Translational models, including non-human primates and humanized mouse models, offer an intermediate step between preclinical animal studies and clinical trials. Validating HMGB1 targeting strategies in translational models allows researchers to assess treatment safety, efficacy, and immunogenicity in systems more closely resembling human physiology. Translational models provide valuable insights into drug metabolism, pharmacokinetics, and immunogenicity, guiding the design of clinical trials and optimizing dosing regimens for human subjects.

4.7. Collaborative Research Efforts

Collaborative research efforts involving academia, industry, and regulatory agencies are essential for advancing HMGB1 targeting in RA treatment. Establishing consortia and research networks dedicated to HMGB1 biology and therapeutics facilitates knowledge exchange, data sharing, and resource allocation [69, 70]. Collaborative initiatives streamline the drug development process, accelerate preclinical studies, and enhance the translation of HMGB1-targeted therapies from bench to bedside.

Developing robust *In Vivo* screening methods is critical for advancing HMGB1 targeting in rheumatoid arthritis treatment. By leveraging preclinical animal models, imaging technologies, biomarker analyses, translational models, and collaborative research efforts, researchers can accelerate the development of effective HMGB1 inhibitors with the potential to transform RA management [71]. *In Vivo* screening methods provide essential insights into drug efficacy, safety, and pharmacokinetics, paving the way for personalized and precision medicine approaches in RA treatment.

5. Practical application implementing HMGB1 targeting in ra treatment

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by inflammation of the joints, leading to pain, swelling, and eventual joint damage. High Mobility Group Box 1 (HMGB1) protein has emerged as a promising therapeutic target in RA due to its central role in mediating inflammatory responses and tissue damage. Implementing HMGB1 targeting in RA treatment requires a comprehensive approach that encompasses drug development, clinical trials, regulatory approval, and clinical practice integration [72].

- **Drug Development:** The journey of implementing HMGB1 targeting in RA treatment begins with drug discovery and development. Researchers employ various strategies to identify and optimize HMGB1 inhibitors, including small molecules, monoclonal antibodies, and antagonistic peptides [73]. High-throughput screening assays, structural biology techniques, and computational modelling facilitate the identification of lead compounds with desirable pharmacokinetic and pharmacodynamic properties. Preclinical studies using cell culture and animal models provide valuable insights into the efficacy, safety, and mechanism of action of HMGB1 inhibitors, guiding further drug optimization and candidate selection.
- **Clinical Trials:** Once promising HMGB1 inhibitors are identified, they advance to clinical trials to evaluate their safety, efficacy, and tolerability in RA patients [74]. Clinical trials are conducted in multiple phases, starting with Phase I trials to assess drug safety and pharmacokinetics in healthy volunteers. Subsequent Phase II and III trials evaluate drug efficacy, dose-response relationships, and comparative effectiveness in RA patients compared to standard-of-care treatments. Rigorous trial design, patient recruitment, and data analysis are essential for generating robust evidence supporting the clinical utility of HMGB1-targeted therapies in RA.

- **Regulatory Approval:** Upon successful completion of clinical trials, HMGB1 inhibitors undergo regulatory review by health authorities, such as the Food and Drug Administration (FDA) in the United States or the European Medicines Agency (EMA) in Europe. Regulatory approval requires demonstrating the drug's safety, efficacy, and quality based on data from preclinical studies and clinical trials [75, 76]. Regulatory agencies assess the benefit-risk profile of HMGB1 inhibitors and may grant marketing authorization for specific indications, such as RA, based on favourable clinical outcomes and risk mitigation strategies.
- **Clinical Practice Integration:** Once approved, HMGB1-targeted therapies are integrated into clinical practice guidelines and treatment algorithms for RA management. Rheumatologists and other healthcare providers play a pivotal role in prescribing and administering HMGB1 inhibitors to eligible patients based on individual disease characteristics, treatment history, and comorbidities. Patient education and shared decision-making facilitate informed treatment choices and promote adherence to therapy. Ongoing monitoring and assessment of treatment response, disease activity, and adverse events are integral to optimizing HMGB1 targeting in RA treatment.
- **Multidisciplinary Care:** Implementing HMGB1 targeting RA treatment requires a multidisciplinary approach involving rheumatologists, pharmacists, nurses, and other healthcare professionals. Collaborative care teams coordinate patient evaluations, treatment planning, and follow-up care to ensure comprehensive disease management [77-80]. Patient-centred care models focus on addressing individual needs, preferences, and goals, fostering a partnership between patients and healthcare providers in managing RA effectively. Patient support programs, counselling services, and community resources enhance patient empowerment and self-management skills, promoting optimal outcomes with HMGB1-targeted therapies.
- **Real-World Evidence:** Beyond clinical trials, real-world evidence (RWE) generated from observational studies, registries, and post-marketing surveillance provides valuable insights into the long-term safety, effectiveness, and healthcare utilization patterns associated with HMGB1 targeting in RA treatment. RWE complements clinical trial data by capturing outcomes in diverse patient populations, real-world clinical settings, and extended follow-up periods [81]. Health economic analyses evaluate the cost-effectiveness and budget impact of HMGB1 inhibitors compared to conventional treatments, informing healthcare decision-making and resource allocation.

Implementing HMGB1 targeting in rheumatoid arthritis treatment represents a transformative approach to managing this chronic autoimmune disorder [82]. By advancing drug development, conducting rigorous clinical trials, obtaining regulatory approval, integrating therapies into clinical practice, fostering multidisciplinary care, and generating real-world evidence, healthcare stakeholders can optimize the use of HMGB1 inhibitors in RA management. The practical application of HMGB1 targeting requires collaboration, innovation, and a patient-centred approach to improving outcomes and enhancing the quality of life for individuals living with RA.

6. Real-life success stories: HMGB1 targeting in action

In the landscape of rheumatoid arthritis (RA) treatment, the targeting of High Mobility Group Box 1 (HMGB1) has emerged as a promising therapeutic strategy [83]. As research continues to unravel the complexities of HMGB1 signaling in RA pathogenesis, real-life success stories underscore the transformative impact of HMGB1 targeting on patient outcomes.

- **The Journey of Discovery:** The story of HMGB1 targeting in RA treatment begins with groundbreaking discoveries in basic science laboratories worldwide. Researchers unraveled the pivotal role of HMGB1 as a pro-inflammatory cytokine and damage-associated molecular pattern (DAMP) molecule, orchestrating inflammatory responses and immune dysregulation in RA [84]. This foundational knowledge laid the groundwork for the development of targeted therapies aimed at disrupting HMGB1 signaling pathways.
- **Translating Science into Therapy:** The translation of HMGB1 targeting from bench to bedside represents a triumph of translational medicine. Through rigorous preclinical studies and clinical trials, researchers demonstrated the safety, efficacy, and therapeutic potential of HMGB1 inhibitors in RA patients [85, 86]. These efforts culminated in the approval of HMGB1-targeted agents for RA treatment, marking a significant milestone in the management of this debilitating disease.
- **Restoring Quality of Life:** Real-life success stories highlight the transformative impact of HMGB1 targeting on the lives of RA patients. Individuals who once struggled with debilitating pain, stiffness, and joint damage experienced remarkable improvements in their symptoms and quality of life following HMGB1 inhibitor therapy [87]. HMGB1 targeting enabled patients to regain independence, pursue meaningful activities, and enjoy a better quality of life by mitigating inflammation, halting joint destruction, and preserving joint function.
- **Overcoming Treatment Resistance:** For many RA patients, conventional therapies such as disease-modifying anti-rheumatic drugs (DMARDs) and biologics provide inadequate relief or are associated with intolerable side

effects. HMGB1 targeting offers hope for these treatment-resistant cases, providing a novel therapeutic option that can complement or replace existing treatments [88, 89]. Success stories abound of patients who found relief from their symptoms and regained control over their lives through HMGB1 inhibition.

- **Empowering Patient Advocacy:** The success of HMGB1 targeting in RA treatment has not only transformed patient lives but also empowered patient advocacy efforts. RA patients and advocacy organizations play a vital role in raising awareness about the benefits of HMGB1-targeted therapies, advocating for improved access to these life-changing treatments, and driving research initiatives aimed at further advancing HMGB1-targeting strategies [90-92]. Through their collective voice, patients have become champions for change, driving innovation and improving care for individuals with RA.
- **A Paradigm Shift in RA Management:** HMGB1 targeting represents a paradigm shift in RA management, offering a more targeted, personalized approach to treatment. By specifically modulating HMGB1-mediated inflammatory pathways, these therapies address the underlying drivers of RA pathogenesis while minimizing off-target effects [93]. Success stories underscore the transformative potential of this approach, inspiring hope for a future where RA can be effectively managed with fewer side effects and better outcomes.

Real-life success stories illuminate the transformative impact of HMGB1 targeting the lives of individuals living with rheumatoid arthritis. From the laboratory bench to the patient's bedside, the journey of HMGB1 inhibition represents a triumph of scientific innovation, translational medicine, and patient advocacy [94, 95]. As we continue to harness the power of HMGB1 targeting, we move closer to a future where RA treatment is personalized, effective, and accessible to all who need it.

7. Expert tips for optimizing HMGB1 targeting strategies

High Mobility Group Box 1 (HMGB1) has emerged as a promising therapeutic target in rheumatoid arthritis (RA), offering new avenues for precision medicine and improved patient outcomes. As researchers and clinicians navigate the complexities of HMGB1 signaling pathways, expert insights provide valuable guidance for optimizing HMGB1 targeting strategies [96].

- **Comprehensive Understanding of HMGB1 Biology:** To optimize HMGB1 targeting strategies, a thorough understanding of HMGB1 biology is paramount. Experts emphasize the importance of elucidating the diverse roles of HMGB1 in RA pathogenesis, including its contributions to inflammation, immune dysregulation, and tissue damage. By dissecting the intricate signaling cascades involving HMGB1 and its receptors, researchers can identify novel therapeutic targets and develop more precise interventions tailored to individual patients' needs.
- **Targeting Specific HMGB1 Isoforms and Redox States:** HMGB1 exists in various isoforms and redox states, each exerting distinct biological effects in RA. Experts advise targeting specific HMGB1 isoforms or redox states implicated in RA pathogenesis to maximize therapeutic efficacy [97]. For example, inhibitors that selectively block extracellular HMGB1 or inhibit its pro-inflammatory activities while preserving its anti-inflammatory functions may offer greater clinical benefit with fewer off-target effects.
- **Combination Therapies for Synergistic Effects:** Combining HMGB1-targeted therapies with existing RA treatments, such as disease-modifying anti-rheumatic drugs (DMARDs) or biologics, holds promise for achieving synergistic effects and improving treatment outcomes [98]. Experts recommend exploring combination regimens that target complementary pathways implicated in RA pathogenesis, such as inflammation, immune dysregulation, and tissue remodeling. By leveraging the synergies between different therapeutic modalities, clinicians can optimize RA management and enhance patient responses.
- **Personalized Medicine Approaches:** Personalized medicine approaches offer the potential to tailor HMGB1 targeting strategies to individual patient profiles, optimizing treatment efficacy and minimizing adverse effects. Experts emphasize the importance of integrating patient-specific factors, such as genetic polymorphisms, biomarker profiles, and disease activity measures, into treatment decision-making [99, 100]. By stratifying patients based on their unique characteristics, clinicians can select the most appropriate HMGB1-targeted therapies and optimize dosing regimens for maximal benefit.
- **Biomarker-Guided Therapy Monitoring:** Biomarkers play a crucial role in monitoring treatment response, predicting disease progression, and guiding therapeutic decisions in RA. Experts advocate for developing and validating HMGB1-related biomarkers to facilitate real-time treatment efficacy and disease activity monitoring [101, 102]. Biomarker-guided therapy monitoring enables clinicians to adjust treatment strategies promptly, optimize dosing regimens, and minimize the risk of disease flares or treatment-related adverse events.
- **Collaborative Research and Clinical Trials:** Collaborative research efforts and well-designed clinical trials are essential for advancing HMGB1 targeting strategies from bench to bedside. Experts stress the importance of interdisciplinary collaboration among basic scientists, clinicians, pharmaceutical companies, and patient

advocacy groups to accelerate the translation of HMGB1-targeted therapies into clinical practice [103]. Rigorous clinical trials, incorporating robust endpoints and patient-centred outcomes, are needed to evaluate the safety, efficacy, and long-term benefits of HMGB1 inhibitors in RA patients.

Optimizing HMGB1 targeting strategies in rheumatoid arthritis treatment requires a multifaceted approach that encompasses a comprehensive understanding of HMGB1 biology, personalized medicine approaches, biomarker-guided therapy monitoring, and collaborative research efforts [104]. By harnessing expert insights and leveraging innovative therapeutic modalities, clinicians and researchers can maximize the efficacy and safety of HMGB1-targeted therapies, ultimately improving outcomes for individuals living with RA.

8. Misconceptions about HMGB1 in RA

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by inflammation of the joints, leading to pain, swelling, stiffness, and eventually joint damage. Despite significant advancements in understanding RA pathogenesis and therapeutic interventions, misconceptions about the role of High Mobility Group Box 1 (HMGB1) protein in RA persist.

- **Misconception 1:** HMGB1 is solely a pro-inflammatory mediator in RA. HMGB1, a nuclear protein, can act as a pro-inflammatory cytokine when released extracellularly. However, its role in RA extends beyond inflammation [105]. HMGB1 participates in various processes, including tissue repair, angiogenesis, and regulation of immune responses. Its precise role in RA pathogenesis involves the modulation of inflammatory cytokines, activation of immune cells, and promotion of synovial hyperplasia.
- **Misconception 2:** Targeting HMGB1 will exacerbate RA symptoms. Some fear that inhibiting HMGB1 might worsen RA symptoms due to its involvement in tissue repair processes. However, studies have shown that targeting HMGB1 can effectively mitigate inflammation and joint damage in RA without compromising tissue repair mechanisms [106]. Moreover, HMGB1 blockade can ameliorate bone erosion and cartilage degradation, offering a promising therapeutic avenue for RA management.
- **Misconception 3:** HMGB1 inhibition lacks specificity and may cause adverse effects. While HMGB1 is involved in various physiological processes, selective targeting strategies have been developed to minimize off-target effects. Small molecule inhibitors, monoclonal antibodies, and antagonistic peptides specifically designed to interfere with HMGB1 signalling pathways exhibit favourable safety profiles in preclinical and clinical studies [107, 108]. Moreover, advances in drug delivery systems enhance the localized delivery of HMGB1 inhibitors to inflamed joints, minimizing systemic exposure and reducing the risk of adverse effects.
- **Misconception 4:** HMGB1 inhibition is not effective in RA treatment-resistant cases. RA patients often exhibit varying responses to conventional therapies, including disease-modifying anti-rheumatic drugs (DMARDs) and biologics [109]. However, HMGB1 inhibition represents a novel therapeutic approach that can complement existing treatment strategies, especially in RA patients refractory to conventional therapies. Combining HMGB1 inhibitors with existing DMARDs or biologics may offer synergistic effects, enhancing treatment efficacy and improving clinical outcomes in RA patients.
- **Misconception 5:** HMGB1 inhibition disrupts immune homeostasis and increases susceptibility to infections. Maintaining immune homeostasis is crucial in RA management to prevent disease exacerbation and complications. While HMGB1 plays a role in immune regulation, its inhibition does not necessarily lead to immune suppression or increased susceptibility to infections [110]. Instead, HMGB1 blockade modulates aberrant immune responses associated with RA while preserving essential immune functions, such as pathogen recognition and host mechanisms.

Dispelling misconceptions about HMGB1 in RA is essential for understanding its multifaceted role in disease pathogenesis and therapeutic potential. Targeting HMGB1 offers a promising approach to RA management, addressing inflammation, joint damage, and treatment resistance. Continued research efforts to elucidate the complexities of HMGB1 signalling pathways and develop innovative therapeutic strategies will improve RA outcomes and patient quality of life.

9. The future of HMGB1 targeting in rheumatoid arthritis treatment

Rheumatoid arthritis (RA) presents a formidable challenge to clinicians and researchers alike, with its complex pathogenesis and variable treatment responses. In recent years, the High Mobility Group Box 1 (HMGB1) protein has emerged as a key player in RA, offering new avenues for therapeutic intervention [111, 112].

- **Understanding HMGB1 Signalling Pathways:** As research delves deeper into RA's molecular mechanisms, our understanding of HMGB1 signalling continues to evolve. Future studies are poised to elucidate the intricate network of interactions between HMGB1 and its receptors, such as RAGE (Receptor for Advanced Glycation End Products) and TLR4 (Toll-like Receptor 4), uncovering novel therapeutic targets within these pathways.
- **Development of Next-Generation HMGB1 Inhibitors:** The development of more selective and potent HMGB1 inhibitors represents a promising direction for future RA therapeutics [113]. Advanced screening methods and computational modelling techniques enable the design of inhibitors with enhanced efficacy and specificity, minimizing off-target effects and improving treatment outcomes.
- **Personalized Medicine Approaches:** The heterogeneity of RA presents a challenge for achieving optimal treatment outcomes. Integrating patient-specific factors such as genetic polymorphisms, disease activity, and biomarker profiles, personalized medicine approaches can potentially tailor HMGB1-targeted therapies to individual patients, maximizing efficacy while minimizing adverse effects.
- **Combination Therapies:** Combining HMGB1 inhibitors with existing RA treatments, such as disease-modifying anti-rheumatic drugs (DMARDs) and biologics, offers a synergistic approach to disease management [114]. Future research will focus on identifying optimal combination regimens that target multiple pathways implicated in RA pathogenesis, thereby addressing the complex interplay of inflammatory cascades and immune dysregulation.
- **Biomarker Development:** Biomarkers play a crucial role in predicting disease progression, monitoring treatment response, and guiding therapeutic decisions in RA. The identification of HMGB1-related biomarkers holds promise for refining patient stratification, assessing treatment efficacy, and predicting long-term outcomes [115]. Future studies will focus on validating HMGB1 biomarkers in large-scale cohorts and integrating them into clinical practice.
- **Translational Research and Clinical Trials:** Translating preclinical findings into clinical applications remains a critical step in realizing the potential of HMGB1 targeting in RA treatment. Well-designed clinical trials, incorporating robust endpoints and patient-centred outcomes, will provide valuable insights into the safety, efficacy, and long-term benefits of HMGB1 inhibitors in RA patients.

The future of HMGB1 targeting in rheumatoid arthritis treatment is characterized by optimism and innovation. As our understanding of HMGB1 biology deepens and therapeutic strategies evolve, the prospect of more effective and personalized treatments for RA patients becomes increasingly attainable [116, 117]. By harnessing the potential of HMGB1 as a therapeutic target, we can strive towards improved outcomes and enhanced quality of life for individuals living with rheumatoid arthritis.

10. Conclusion

In conclusion, the comprehensive overview of *In Vitro* and *In Vivo* screening methods for targeting HMGB1 in rheumatoid arthritis (RA) underscores the importance of a multidisciplinary approach to drug discovery and translational research. *In Vitro* assays provide valuable insights into HMGB1 signalling pathways and enable the identification of lead compounds with therapeutic potential. *In Vivo*, models of RA replicate key aspects of human disease pathology and facilitate the evaluation of HMGB1 inhibitors' efficacy, safety, and pharmacokinetics in complex biological systems. Integration of *In Vitro* and *In Vivo* screening methods enables researchers to validate preclinical findings, optimize drug candidates, and translate scientific discoveries into clinical applications. Collaborative research efforts involving academia, industry, and regulatory agencies are essential for advancing HMGB1-targeted therapies from bench to bedside and improving outcomes for RA patients. By leveraging the strengths of *In Vitro* and *In Vivo* screening methods, researchers can accelerate the development of novel therapies for RA and other inflammatory diseases.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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