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Targeting IRAK4 kinase in autoimmune diseases and cancer

Regulace kinázy IRAK4 v léčbě autoimunitních onemocnění a rakovině

Bachelor's thesis

Supervisor: Mgr. Peter Dráber, Ph.D.

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Podpis

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Abstract

The immune system provides the host with protection against invading pathogens. However, its aberrant activation can lead to the development of autoimmune diseases or cancer. Understanding the mechanisms of inflammation and immune responses is crucial for the treatment of such conditions and reestablishing immune balance. Toll-like receptors and interleukin-1 family receptors are key components of the innate immune system. Proteins MyD88 and IRAK4 are essential for the function of both receptor families and their deficiency causes host susceptibility to infection. On the other hand, overactivation of signaling pathways employing MyD88 and IRAK4 was shown to promote autoimmunity and cancer. The main focus of this text will be to summarize current knowledge about the mechanism of IRAK4 signaling and how it can be exploited in the development of therapeutics.

Keywords

IRAK4, MyD88, Toll-like receptors, IL-1 receptor, cytokines, autoimmunity, cancer

Abstrakt

Imunitní systém poskytuje organismu nezbytnou ochranu před patogeny. Pokud se ale vymkne kontrole, může mít destruktivní důsledky vedoucí k rozvoji autoimunitních onemocnění nebo rakoviny. Porozumění mechanismům vzniku zánětu a imunitních odpovědí je zásadní pro léčbu těchto onemocnění a obnovu imunitní rovnováhy. Toll-like receptory a receptory rodiny receptoru interleukinu 1 jsou klíčovou součástí vrozené imunity. Obě receptorové rodiny využívají k signalizaci proteiny MyD88 a IRAK4 a jejich deficience způsobuje větší náchylnost k infekcím. Na druhou stranu bylo dokázáno, že přehnaná aktivace těchto signálních drah může podnítit vznik autoimunitních onemocnění a přispívat k rozvoji rakoviny. Hlavní zaměření této práce je shrnutí dosavadních poznatků o mechanismu signalizace kinázy IRAK4 a jak můžou být tyto znalosti využity k vývoji léků.

Klíčová slova

IRAK4, MyD88, Toll-like receptory, IL-1 receptor, cytokiny, autoimunita, rakovina

Contents

List of abbreviations

AP-1	activator protein 1
DD	death domain
ERK	extracellular signal-regulated kinase
IFN	interferon
IKK	IκB kinase
IL	interleukin
IL-1R	IL-1 receptor
IRAK	interleukin 1 receptor-associated kinase
IRF	interferon regulatory factor
JNK	c-Jun N-terminal kinase
K48-Ub	Lys48-linked ubiquitin
K63-Ub	Lys63-linked ubiquitin
KD	kinase dead, kinase inactive
LUBAC	linear ubiquitin chain assembly complex
M1-Ub	linear ubiquitin, Met1-linked ubiquitin
MAPK	mitogen-activated protein kinase
MKK	mitogen-activated protein kinase kinase
MS	multiple sclerosis
MyD88	myeloid differentiation primary response 88
NEMO	NF-κB essential modulator
NF-κB	nuclear factor κB
PROTAC	proteolysis targeting chimera
RA	rheumatoid arthritis
SLE	systemic lupus erythematosus
TAK1	transforming growth factor β-activated kinase 1
Th	helper T cell
TIR	Toll/interleukin 1 receptor
TLR	Toll-like receptor
TRAF	tumor necrosis factor receptor-associated factor

1. Introduction

The immune system is essential for host protection against infection and can prevent the development of cancer. However, if left unchecked, it can have deleterious effects on the host's body and cause autoimmunity. As insufficient activation and overactivation are both harmful, a tight balance between the two must be maintained.

In mammals, the immune system comprises of two distinct but intertwined systems: innate and adaptive immunity. The innate immune system is the body's first line of defense against infection. It recognizes a broad spectrum of microbial components (e.g., parts of bacterial cell wall, nucleic acids, metabolites) generally referred to as pathogen-associated molecular patterns (PAMPs) and body's own molecules, whose presence outside of cells indicates cell damage, so called damage-associated molecular patterns (DAMPs). The innate immune system is sometimes described as non-specific because the recognized molecules are not exclusive for a certain microbe, but rather evolutionarily conserved across genera. The main cells of the innate immune system are dendritic cells, granulocytes, macrophages, monocytes, and natural killer (NK) cells and are mobilized within minutes of infection. On the other hand, the adaptive immune system is modulated after the encounter with a pathogen and develops to combat the specific infectious agent. The cells involved are T and B lymphocytes, their development is accompanied by genetic rearrangement and clonal selection, and its maturation can take several days. The mature cells can then recognize and target a particular pathogen or infectious agent with high specificity [1, 2].

Upon PAMP or DAMP recognition, cells of the innate immune system start producing a range of molecules to fight the infection, attract effector cells (NK cells, neutrophils, or macrophages), and activate the adaptive immune system. An important class of molecules used by cells to communicate with each other during immune responses are cytokines. Cytokines are small proteins produced by various cells, immune and non-immune, to modulate inflammation. They can work locally on nearby cells or enter the bloodstream and affect distant cells. The general purpose of cytokines is to recruit immune cells to the site of inflammation, help combat the infection, and promote the healing of damaged tissue [3]. Cytokines can be divided into groups with interleukins (IL) forming the largest category. The majority of interleukins are produced by T lymphocytes and they have various biologic functions e.g., to regulate and direct inflammation, and influence leukocyte maturation [4].

The focus of this text will be the description of the main receptors involved in the recognition of infectious agents, Toll-like receptors and interleukin-1 receptors, their engagement in host protection and disease with emphasis on their downstream signaling, and the potential of targeting this pathway in disease treatment.

Aims of this thesis

1. summarizing current knowledge about IL-1 family and Toll-like receptors and their signaling
2. describing the key downstream proteins MyD88 and IRAK4
3. evaluating the potential of IRAK4 in disease therapy
4. reviewing current trends in IRAK4 targeted drug design

2. The role of IL-1 and Toll-like receptor family in immunity

2.1. IL-1 family cytokines and receptors

Interleukin-1 (IL-1) family cytokines promote inflammation in a broad sense. They induce fever, mediate leukocyte infiltration, T cell activation, and stimulate production of acute phase proteins and other proinflammatory cytokines [5]. IL-1 family members are expressed ubiquitously across all cell types. IL-1 family includes seven proinflammatory cytokines: IL-1α/β, IL-18, IL-33, IL-36α/β/γ, and four cytokines with anti-inflammatory function: IL-1Ra, IL-36Ra, IL-37, IL-38 [6]. They each bind to their respective receptors which will be described below. Most members of the family are produced as a precursor that needs to be cleaved in order to yield the biologically active form. For IL-1β and IL-18 the cleavage occurs in an inflammasome-caspase 1-dependent manner [7, 8]. Others are processed by various enzymes induced in inflammatory microenvironment. IL-1 cytokines are intracellular, and the precursor is usually localized in the nucleus, except for IL-1Ra, which contains secretory signal peptide and is secreted. The presence of IL-1 cytokines in the extracellular space is generally associated with cell damage [9].

Receptors of the IL-1R family are cell surface receptors that follow a general structure containing three extracellular immunoglobulin-like (Ig-like) domains participating in ligand binding, a transmembrane domain, and an intracellular Toll/IL-1 receptor (TIR) domain responsible for signal transduction [10-13]. Over the years, ten receptors for IL-1 family cytokines have been described, in some cases repeatedly, resulting in multiple names for the same molecule. A unifying nomenclature based on numbering (IL-1R1 – IL-1R10) has been proposed in a review by Boraschi and Tagliabue [14] and will be used further in this text. The receptor family can be divided into three groups based on their function. (i) Receptors: IL-1R1, IL-1R2, IL-1R4, IL-1R5, IL-1R6, that are unique for each proinflammatory IL-1 family cytokine. (ii) Co-receptors: IL-1R3, IL-1R7, usually shared by multiple members of the IL-1 family. (iii) Orphan receptors: IL-1R8, IL-1R9, IL-1R10, with largely unknown mechanisms of action.

IL-1R1 binds the founding members of the IL-1 cytokine family, IL-1α and IL-1β, as well as the antagonistic IL-1Ra. Signaling of this receptor was the first IL-1 receptor to be described and is currently the best understood of the family. It induces transcription of proinflammatory genes and is a factor in maturation of a subset of cytokine producing CD4+ T helper cells (Th), particularly Th17 [15]. IL-1R2 lacks the cytoplasmic TIR domain, which renders it unable to

signal, and serves as a decoy receptor for IL-1α/β. It exerts anti-inflammatory function by direct competition for ligand with the pro-inflammatory IL-1R1 [16, 17]. IL-1R3 is a co-receptor utilized by IL-1R1, IL-1R2, IL-1R4, and IL-1R6. IL-1R4 is the receptor for IL-33 and plays a role in Th2 activation and differentiation [15]. IL-1R5, along with its unique co-receptor IL-1R7, binds IL-18 and is expressed in larger quantities in activated Th1 cells. IL-1R6 binds IL-36 and has been proposed to form activating feedback loop in psoriasis. IL-1R8 was proved to have anti-inflammatory effects through inhibition of other IL-1R downstream signaling [18]. For IL-1R9 and IL-1R10, there are currently no known ligands. It has been observed, nonetheless, that mutations in these receptors are connected with X-linked mental retardation. [19-21]. Overall, IL-1Rs have a vital role in shaping the adaptive immune response following infection or injury.

Signaling through IL-1R1 is the prototype of IL-1 signaling and with slight variations can be applied to all IL-1 cytokine and receptor family members. First, IL-1α/β binds to the IL-1R1 monomer and associates with IL-1R3. Then their extracellular (Ig-like) domains dimerize, which in turn causes dimerization of their intracellular TIR domains, recruitment of crucial downstream adaptor myeloid differentiation primary response 88 (MyD88), and signal transduction (Fig. 1). The dimerization of TIR domains is critical for the initiation of signaling as truncated receptors were unable to signal. Similarly single receptor chain, without a co-receptor, is insufficient for signal transduction, as seen in the mechanism of some receptor antagonist action (IL-1Ra, IL-37Ra) [22].

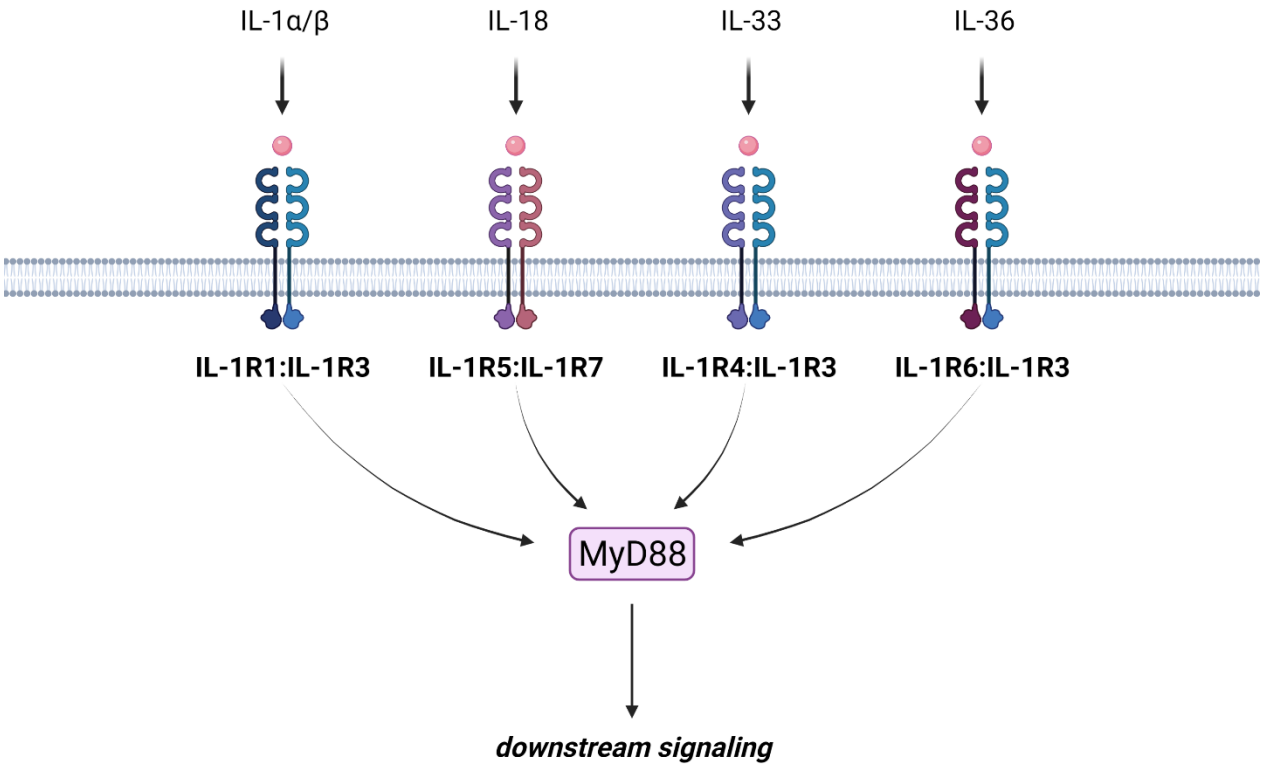

Figure 1. IL-1 family receptors with ligands. *IL-1 family receptors associate with coreceptors after binding their respective ligands. All these receptors recruit adaptor protein MyD88 to initiate downstream signaling. Created with BioRender.com*

2.2. Toll-like receptors

TLRs are a class of pattern recognition receptors (PRRs) which recognize a wide variety of pathogen associated molecular patterns (PAMPs), as well as host-derived damage associated molecular patterns (DAMPs). To date, ten TLRs have been identified in humans: TLR1-10. While in mouse, twelve have been discovered: TLR1-13, with TLR10 being a pseudogene. TLR1, TLR2, TLR4-6, and TLR10 are mostly localized on the surface of cells, though TLR4 is fairly abundant both on cell surface and in endosomes. Their ligands are mainly components of extracellular bacteria and their metabolites. TLR3, TLR7-9, and TLR11-13 are primarily in endosomes. Endosomal TLRs are largely sensors of nucleic acids. TLRs are expressed in leukocytes, fibroblasts, and epithelial cells in various combinations [23, 24]. All TLRs contain three distinct domains. An N-terminal LRR (leucine-rich repeat) domain necessary for ligand recognition, a transmembrane domain, and a C-terminal TIR domain [25].

Upon ligand binding, TLRs form homodimers, as is the case for TLR3-5 and TLR7-13, or heterodimers, in the case of TLR1:2 and TLR2:6 [26]. Similar to the IL-1 receptors, formation of receptor dimers leads to dimerization of their intracellular TIR domains, which enables the recruitment of adaptors MyD88 or TRIF and initiation of downstream signaling (Fig. 2).

TLRs are core components of the immune system. Deficiency in TLR signaling leads to higher incidence of infections, while their aberrant activation is one of the driving forces behind inflammatory bowel disease (IBD), allergy, asthma, and sepsis.

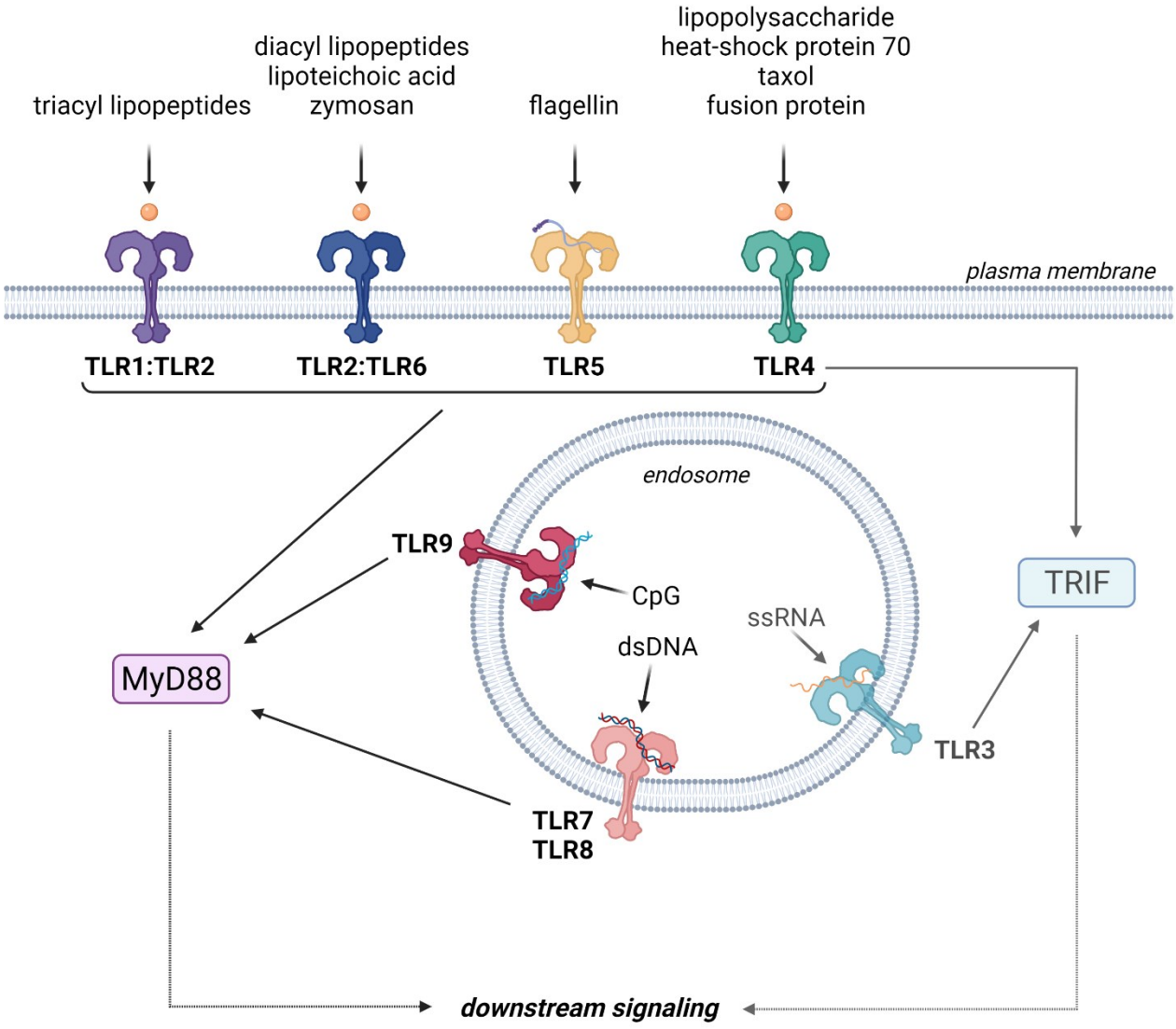

Figure 2. Toll-like receptors and their ligands: *Plasma membrane and endosomal TLRs with their respective ligands. Upon ligand binding, TLRs dimerize which leads to recruitment of adaptor proteins MyD88 or TRIF and downstream signaling initiation. Created with BioRender.com*

2.3. IL-1 receptor/Toll-like receptor superfamily

Together, IL-1Rs and TLRs belong to a superfamily defined by the presence of a highly conserved TIR domain (IL-1R/TLR superfamily) (Fig. 3). This family is not exclusive to receptors and also encompasses an array of adaptor proteins. Similar sequence motif has been identified in a wide variety of organisms ranging from plants to mammals, highlighting its evolutionary importance. The convergence of signaling pathways of a cytokine receptor and a pattern recognition receptor represents an important link between the innate and adaptive immune system and may shed some light on how the adaptive immune system emerged and how it is regulated.

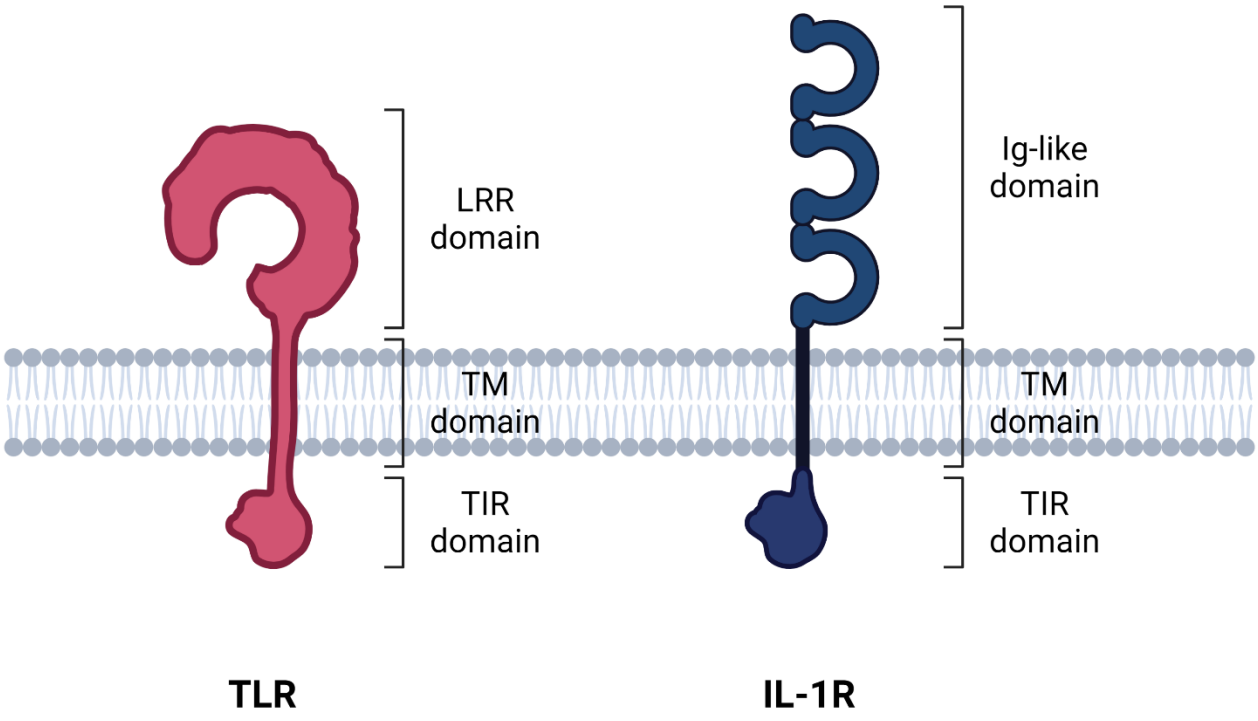

Figure 3. Comparison of TLR and IL-1R structure: *While the extracellular domains of these receptors differ significantly, their intracellular TIR domains are highly conserved. When TIR domains dimerize, they form a docking platform for other TIR domain-containing proteins. Created with BioRender.com*

There are five main TIR domain-containing adaptor proteins that mediate signal transduction from the receptor to effector molecules: (i) Myeloid differentiation primary response 88 (MyD88), the first TIR domain-containing adaptor to be described, is utilized by most Toll/IL-1 receptors, namely IL-1R1, and IL-1R3-7 and all TLRs apart from TLR3 [27]. (ii) MyD88 adapter-like protein (MAL, also known as TIRAP) is involved in TLR2 and TLR4 signaling as the first receptor interactor that subsequently binds MyD88 [28]. (iii) TIR domain-containing adapter inducing interferon β (TRIF, also called TICAM1) is the exclusive adaptor used by TLR3 and in alternative TLR4 pathway to induce transcription of type 1 interferons [29]. (iv) TRIF-related adaptor molecule (TRAM, also referred to as TICAM2) interacts with TLR4 as a platform for TRIF recruitment and has been proposed to function in bringing MyD88 to IL-1Rs [30]. Lastly, sterile α and TIR motif-containing protein 1 (SARM) serves as a negative regulator of MyD88 and TRIF downstream signaling [31].

MyD88 is an adaptor employed by the majority of Toll-like and IL-1 receptors and its importance and signaling will be discussed in greater detail further in the text.

2.4. Patients with disrupted in IL-1 and Toll-like signaling pathways

Even though downstream signaling pathways of IL-1 and Toll-like receptors converge, their disruption leads to a broad spectrum of diseases. Some are influenced mostly by one of the receptor family, in others both receptor families contribute to disease pathology.

Since Toll-like receptors are a pivotal component of the immune system when it comes to recognizing invading pathogens, deficient signaling of these receptors leads to increased susceptibility to microbial infections [32]. Genetic variations in TLRs have been intensely studied in relation to bacterial and viral susceptibility. One of the first described were polymorphisms in TLR4 associated which increased sensitivity to Gram-negative bacteria and patients bearing these mutations are at a higher risk for septic shock [33]. In the following years, a myriad of SNPs in all TLRs were described, many of them linked to common diseases of the industrialized world, such as IBDs, allergy, and asthma. However, studies focusing on TLR polymorphisms vary significantly based on population size and background and should be approached with caution [34, 35]. Much more solid evidence for TLR involvement is in primary immunodeficiencies (PIDs) with defects in the innate immune system. Among the most common defects in PIDs are those of MyD88 and IRAK4, leading to susceptibility to pyogenic bacteria, most notably *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Of note is that even though bacterial infections in patients deficient in TLRs present often and have high mortality at young age, in adulthood the number and severity of infections is substantially decreased [36]. This indicates a crucial role of TLRs in providing immune protection during first encounter with a pathogen, before the adaptive immune system is fully developed [37].

A range of conditions is influenced by both IL-1 and Toll-like receptor signaling. Bellow, the most common are discussed.

Rheumatoid arthritis (RA) is a systemic autoimmune disease that usually first manifests between 25 and 45 years of age and presents with swollen joints, cartilage destruction, and bone erosion. IL-1 contributes to RA progression by stimulating synovial cells to release collagenases and prostaglandins, and by mediating leukocyte infiltration into synovial tissue, resulting in progressive cartilage degeneration and increasing joint pain [38]. Interestingly, TLR expression is elevated in RA synovial tissue compared to healthy control. However, it is not clear whether this is a cause or the consequence of RA inflammation. A recent study suggested ligation of non-coding

miRNA to TLR7 is the trigger for RA onset [39]. It is apparent, though, that TLRs play a role in perpetuation of the disease as they are able to induce NLRP3 inflammasome activation and subsequent IL-1β processing and release [40]. Prolonged systemic inflammation in RA patients can lead to comorbidities that severely decrease the quality of life, most notably cardiovascular disease [41]. IL-1 is suspected in other arthritic conditions, such as gout, where the presence of uric crystals in combination with fatty acids in the synovium triggers IL-1 production, and osteoarthritis, caused by increased chondrocyte apoptosis [42, 43].

TLR/IL-1R signaling appears to be driving the disease progression in psoriasis, a skin disease with a prevalence of around 2.5% in Caucasian population and usual onset before the age of 40 [44]. It is characterized by pink-red skin plaques with scales of dead skin and around 23 % of the patients also suffer from arthritis [45]. Psoriatic patients usually go through cycles of flare-ups, with increased skin lesion size and quantity, and remission. Histology analysis of skin lesions revealed morphological abnormalities like thickened epidermis, thinning in the protective granular layer, and T cell infiltration to the dermis and epidermis [46]. Even though psoriasis is considered a T cell-mediated (especially Th1 and Th17) disease, TLRs are suggested to be responsible for the initiation. In support of this are observed bacterial infections and tissue injury prior to psoriasis development or condition worsening [47]. A change in the skin microbiome composition in psoriatic skin has also been detected [48]. TLR-mediated cytokine production upon PAMP or DAMP recognition may be the primary impulse for psoriasis development in predisposed individuals. Increased levels of IL-1β in lesional skin were also reported, which may contribute to the pathological activation of Th17 [49]. Additionally, mutations in the gene coding IL-36 are associated with pustular psoriasis, a rare form of psoriasis [50, 51].

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease affecting around 0.013-7.7 % of global population, depending on the region, with higher incidence risk in women [52]. The most common clinical manifestations of SLE are rash (with photosensitivity), swollen and painful joints, kidney disorders, often accompanied with pleuritis and pericarditis [53]. However, SLE can affect virtually any organ. Antibody producing B cells in SLE patients show increased expression of TLR7 and higher levels of circulating IL-18 were detected in comparison to healthy individuals [54, 55]. Furthermore, one murine model of SLE is induced through overexpression of TLR7, suggesting it plays a core role in the development of SLE [56].

Interestingly, TLR7 gene is located on the X chromosome and was proved to be able to escape dose dependent inactivation [57]. This phenomenon would explain higher incidence of SLE in women.

Multiple sclerosis (MS) is an autoimmune disease of central nervous system where the host's body, most prominently autoreactive CD4+ T cells, attacks the protective myelin tissue around axons of neurons. The resulting symptoms are fatigue, impaired cognitive function, loss of mobility, and ataxia [58]. The first signs of the disease usually start occurring in early adulthood and continue to worsen in time. Since MS affects patients in the peak of their productive life, this disease inflicts huge psychological and economic hardship on patients and their families. Global prevalence of MS is 44 per 100.000 population in 2020 with an increasing tendency, probably due to earlier diagnoses and increased survival because of improved therapies [59]. Most of the evidence on IL-1R and TLR involvement comes from an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). To state a few, mice deficient in IL-1R1 failed to develop EAE upon induction and TLR expression was significantly increased in mice during disease progression [60]. It has also been found that TLR2 signaling can shift regulatory T cells, critical for self-tolerance, towards an inflammatory Th17 phenotype [61, 62]. The study of TLRs in the context of MS is underway with a number of TLR aimed therapies in clinical trials [63].

Adult onset Still's disease (AOSD) and systemic onset juvenile idiopathic arthritis (sJIA) fall under the category of autoinflammatory diseases. Patients exhibit similar symptoms of joint pain and swelling, and recurrent fever and rash. Direct mechanism of pathogenesis remains unclear. Patients usually do not respond to immunosuppressant treatment, however, IL-1 blockade provides long-term improvement in most [64].

Impairment of IL-1 signaling has primarily been associated with autoimmune and autoinflammatory diseases. However, blocking of this pathway has emerged as a promising target in lessening the severity of several conditions, where inflammation is not the main cause, such as type 2 diabetes [65]. In type 2 diabetes, increased IL-1 production, most notably IL-1β, contributes to higher amount of apoptosis of pancreatic β cells, general impairment of their secretory function and can even induce insulin resistance [66]. Furthermore, when patients were treated with recombinant IL-1 receptor antagonist (anakinra), or monoclonal antibodies directed at IL-1β, their condition improved [67]. There is a growing body of evidence suggesting the employment of IL-1

in other pathological processes. It has been reported that anakinra administration can decrease adverse tissue remodeling after myocardial infarction and slow down the progression of neurodegenerative diseases like amyotrophic lateral sclerosis and Alzheimer's disease [68, 69]. However, these data come from *in vitro* studies and animal models, and the translation into clinic has not been very successful so far, with significant variations in treatment responsiveness. This suggests a more complex mechanism of pathology in human patients.

Cancer is an overarching term for a large group of conditions where host's own cells start uncontrollable growth and proliferation. Tumorigenesis can be influenced by many factors both genetic and environmental. Prolonged state of inflammation can lead to changes in cell regulatory processes and increase the risk of tumor development. IL-1 family cytokines have been proven to have several functions in the tumor microenvironment. They promote angiogenesis, macrophage infiltration, can induce immunosuppressive phenotype in natural killer cells causing immune system evasion by the tumor, and plays a role in cancer metastasis, to list a few [70-72]. IL-1 cytokines are also among the molecules abundantly produced by tumor cells. While IL-1R signaling axis has mostly pro-tumorigenic effects, the situation is much more complicated in Toll-like receptors. TLRs have been proven to have both protumor and antitumor effects, mostly depending on the type of tumor and affected cell type. Among the antitumor effects are induction of apoptosis in cancer cells, potentiation of the effects of chemotherapy, as well as induction of type I interferons which inhibit tumor growth [73, 74].

2.5. Targeting IL-1R and TLR in disease

Treatment of PIDs is generally aimed at combating acute infection with antibiotics or treatment with prophylactic antibiotics. Nowadays, there are new emerging TLR antagonism-based therapies for both PIDs, mainly oriented at TLR7 and TLR9, and autoimmune diseases, targeting the whole spectrum of TLRs. However, TLR downstream molecules are being explored much more in terms of therapy development [75].

The first treatment of inflammatory diseases is usually with non-steroidal anti-inflammatory drugs such as ibuprofen or aspirin, or glucocorticoids. However, these drugs irritate the digestive system, can cause bleeding and long-term use can even lead to immunosuppression. More targeted approach is therefore beneficial. Tactics for IL-1 mediated disease treatment are directed at preventing its production by the inflammasome through the inhibition of caspase 1, or receptor binding, which includes recombinant IL-1 receptor antagonist (anakinra), monoclonal anti-IL-1β antibody (canakinumab), and anti-IL-1R1 antibody. The most widely used therapeutic targeting IL-1R signaling is anakinra. This is in part due in large to its record safety. Anakinra has been used extensively in many conditions with suggested IL-1 involvement ranging from psoriasis to various types of cancer [65].

3. MyD88 as a major signaling molecule

MyD88 was first identified as a gene induced in murine myeloid cells following IL-6 stimulation and as a marker of their terminal differentiation [76]. It was later described to be a component of the IL-1 and Toll-like receptor complexes [27]. MyD88 is a cytoplasmic adaptor protein containing a C-terminal TIR domain by which it interacts with the receptor, an intermediate linker domain (ID), and an N-terminal death domain (DD) which recruits downstream signaling molecules (Fig. 4) [77].

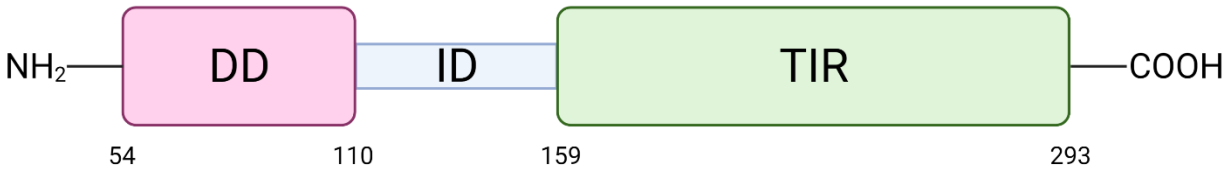

Figure 4. Schematic structure of MyD88: *DD death domain, ID intermediate domain, TIR Toll/IL-1 receptor domain. Created with BioRender.com*

3.1. Signalosome

After ligand is bound to Toll-like and IL-1 receptors, their TIR domains dimerize and create a nucleation site for other TIR domain-containing proteins. For most Toll/IL-1 receptors the adaptor involved is MyD88. There is an exception in the case of TLR4 and TLR2 where MyD88 does not interact with the receptor directly but via TIRAP that serves as a bridge between the two. Subsequently, when MyD88 is recruited to the receptor, its DD facilitates the docking of DD-containing interleukin-1 receptor-associated kinase (IRAK) kinases. While IRAK1 and 2 are somewhat redundant in their action and dispensable for signaling, IRAK4 is integral to proper signalosome assembly and function, supported by only partial disruption of signaling in the absence of IRAK1 compared to complete ablation when IRAK4 is missing [78].

MyD88, IRAK4, and IRAK1/2 assemble to compose a closed helical complex known as the myddosome [79]. The complex forms in a hierarchal order through DD clustering. First, MyD88

and IRAK4 form a stable intermediate consisting of six MyD88 and four IRAK4 molecules. IRAK4 undergoes autophosphorylation and activation. Activated IRAK4 is then able to bind four molecules of IRAK1/2 to form the whole complex. IRAK1/2 is subsequently phosphorylated and activated by IRAK4 [80]. After activation, IRAK1/2 engages E3 ubiquitin ligases, enzymes, which catalyze the addition of ubiquitin chains to themselves and other proteins. Apart from the ubiquitin chains linked through Lys48 (K48) that mark proteins for proteasomal degradation, there are other types of ubiquitin chains with scaffold function, such as Lys63- and linear Met1-linked ubiquitin chains (K63-Ub, M1-Ub). The first E3 ubiquitin ligases to be recruited to the myddosome are Pellino1, activated via IRAK-catalyzed phosphorylation, and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), which interacts with the C-terminal of IRAK1/2 [81, 82]. Both proteins generate K63-linked ubiquitin chains, and their function in the MyD88 pathway is to some extent redundant [83]. K63-Ub facilitates the binding of another ubiquitin ligase, linear ubiquitin chain assembly complex (LUBAC), the only enzyme known to generate M1-linked ubiquitin chains [84, 85]. These enzymes cooperate to create a complex network of K63, M1 or hybrid K63/M1 ubiquitin chains that provide a docking platform for various proteins and protein complexes. Transforming growth factor β (TGFβ)-activated kinase 1 (TAK1) is a master kinase that initiates two major signaling pathways: the nuclear factor κB (NF-κB) pathway, and the mitogen-activated protein kinase (MAPK) cascade. TAK1 activation is dependent on K63-Ub binding which is mediated by TAK1-binding protein 1 and 2 (TAB1/2) [86]. The absence of K63-Ub or mutated ubiquitin binding motif of TAB2, severely impairs or completely abolishes TAK1 action [87]. Once bound to K63-Ub, TAK1 is able to undergo autophosphorylation in the activation loop and gain its full function [88]. Another kinase participating in activation of both the NF-κB and MAPK pathways is MEKK3. The recruitment and activation of MEKK3 is likely catalyzed by TRAF6 [89].

Also recruited to the ubiquitin network is the IkB kinase (IKK) complex that consists of three subunits: IKKα, IKKβ, and NF-κB essential modulator (NEMO, also known as IKKγ). IKKα/β are catalytical subunits, while NEMO has a regulatory function. NEMO binds to both K63-Ub and M1-Ub, though the affinity for M1-Ub is approximately 100-fold higher, and brings the kinase complex to the receptor to be activated [90]. IKKβ is phosphorylated by TAK1 followed by IKK autophosphorylation [91]. This leads to rapid phosphorylation of IκB which is then marked with K48-Ub for degradation by the proteasome. Once IκB is degraded, the transcription factor NF-κB

is free to translocate into the nucleus and initiate the transcription of proinflammatory cytokines and chemokines, such as IL-6, IL-8, TNFα, and CXCL1 [92]. A TAK1-independent way of NF-κB activation mediated by MEKK3 consists of NEMO phosphorylation which leads to IKKα activation. IKKα then phosphorylates IκB, causing it to dissociate from NF-κB without degradation [93].

The MAPK pathway is important for cellular response to extracellular stimuli and results in changes in cell proliferation, differentiation, or cell death. The cascade begins with activation of MAPK kinase kinase (MAPKKK) upon recognition of extracellular cues. MAPKKK then relays the signal to MAPK kinase through phosphorylation. In mammals, the cascade branches into three parts characterized by the final activated MAPK family kinase: cJun N-terminal kinase (JNK), p38, extracellular signal-regulated kinase (ERK) [94]. In IL-1R/TLR downstream signaling the prime targeted MAPKs are JNK and p38.

The JNK family kinases respond to environmental stress signals, growth factors, and inflammatory cytokines. Their upstream activators are primarily MKK4 and 7. Once activated, they interact with factors that compose the transcription factor AP-1 and subsequently induce proinflammatory cytokine gene transcription [95].

p38, similarly to the JNK kinases, react strongly to external stress stimuli and inflammatory cytokines. p38 is primarily activated by MKK3 and 6, although phosphorylation by MKK4 has been reported. The downstream targets of p38 include factors involved in cell proliferation and apoptosis [96].

TAK1 functions as MAPKKK in the activation of JNK and p38 pathways by phosphorylating MKK4/7 and MKK3/6, respectively [89, 91]. This, along with the activation of the NF-κB pathway, initiates the transcription of proinflammatory genes and modulates the immune response to IL-1R/TLR stimulation (Fig. 5).

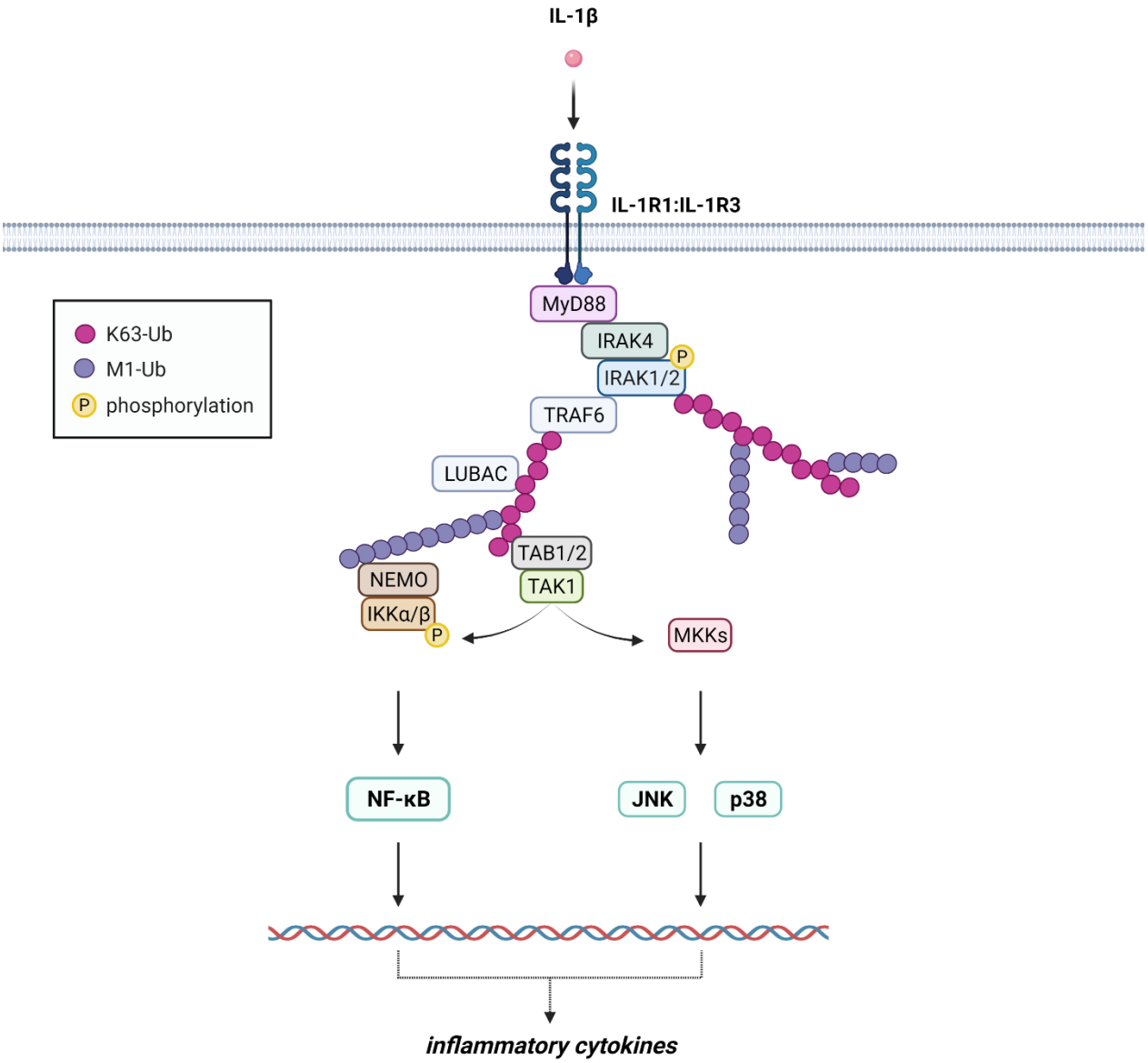

Figure 5. IL-1R1 receptor downstream signaling: *IL-1R1 signaling displays the prototypical downstream activation shared by all TIR-domain containing receptors employing MyD88. When ligand is bound to the receptor and its TIR domains dimerize, rapid assembly of the myddosome is initiated. MyD88, IRAK4 and IRAK1/2 are sequentially recruited to the receptor. Activated IRAK1/2 engages TRAF6 which along with LUBAC starts building molecular scaffold of non-degradative polyubiquitin chains. These chains serve as a docking platform for TAK1 and the IKK complex. TAK1 interaction with the IKK complex and MAPKKs results in transcription of proinflammatory genes. Created with BioRender.com*

3.2. MyD88 deficiency

Even though MyD88 is an adaptor downstream of quite a large number of receptors, surprisingly, deficiency in this protein leads to susceptibility to infection by a relatively narrow range of pyogenic bacteria in humans [97]. However, these infections are life threatening and many patients do not survive to adulthood. The severity and frequency of infections tends to decrease with age and adult patients can live healthily without the need of prophylactic antibiotic treatment. These deficiencies are often caused by in-frame deletions or missense mutations in the intermediate domain (ID) or the DD [36]. A splice variant of MyD88 completely lacking the ID, MyD88s, was shown to act as a dominant negative inhibitor of IL-1β and LPS induced NF-κB activation [98]. The ID, specifically a few residues close to the DD (Gln114-Leu118), is indispensable for homotypic interactions with other MyD88 molecules, IRAK4 recruitment, and consequent myddosome formation [99].

In mice, genetic knockout of MyD88 abrogated the induction of Th1 target genes after IL-18 stimulation as well as the production of cytokines following LPS challenge [100, 101]. Nonetheless, MyD88 KO rescued autoimmune phenotype in many murine models of inflammation [102]. Moreover, MyD88 KO mice show lower incidence of graft-versus-host disease, implying MyD88 plays a part in transplant immunity [103].

On the other hand, overactivation in chronic inflammation and gain-of-function mutations of MyD88 can result in constitutive activation of the NF-κB/AP-1 pathway and, eventually, malignant transformation, particularly B cell malignancies [104].

Several compounds targeting MyD88 have been developed, mostly small molecule inhibitors. In general, MyD88 inhibitors aim at disruption of its interaction with other molecules, the TIR domain interaction with receptors and the DD interaction with other MyD88 molecules and IRAKs. Additionally, mRNA silencing is also used as a mean of MyD88 inhibition [102]. However, since MyD88 is an adaptor protein, designing effective inhibitors is complicated. Focusing on the kinase directly downstream MyD88, IRAK4, has a greater potential of successful drug design.

4. IRAK4 as a critical regulator of signaling

IRAK4 was discovered as the fourth and last member of the structurally related IRAK kinases. IRAKs belong to a group of Ser/Thr protein kinases which elicit their activity by phosphorylating -OH residues of serin and threonine. Despite extensive studies of IRAKs, uncertainties about their function remain to this date. It is clear, however, that IRAK4 plays a major role in the myddosome assembly and ensures proper signal transduction [78].

4.1. Molecular structure of IRAK4

IRAK4 has an N-terminal DD and central kinase domain linked by an unstructured proline/serine/threonine-rich region. In contrast with other IRAK family proteins, the kinase domain of IRAK4 is followed by only few residues, while IRAK1, IRAK2, and IRAK-M have a long C-terminus (Fig. 6). Crystal structure of IRAK4 suggests a possible function as a tyrosine kinase in addition to Ser/Thr, since IRAK4 contains motifs present in many tyrosine kinases. However, no tyrosine kinase activity has been detected in IRAK4 so far. Several residues were assigned as crucial in different IRAK4 biologic activities. Phosphorylation of Ser8 of its DD influences myddosome assembly, phosphorylated Thr345 in the activation loop is a sign of IRAK4 activation. Lys213 is the catalytic residue in the central kinase domain and its substitution with alanine results in kinase inactive IRAK4 [105].

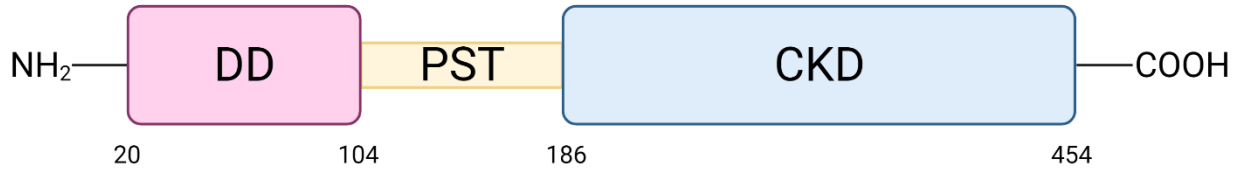

Figure 6. Schematic structure of IRAK4: *DD death domain, PST proline/serine/threonine-rich region, CKD central kinase domain. Created with BioRender.com*

IRAK4 works in two ways in the myddosome and shapes IL-1R/TLR downstream signaling in a kinase-independent and kinase-dependent manner. As mentioned above, myddosome assembly is initiated by receptor TIR domain dimerization followed by MyD88 recruitment. In the absence of IRAK4, MyD88 clusters into large receptor-associated oligomers with varying lifespan. In comparison, chemical inhibition of IRAK4 kinase activity does not cause such large MyD88 oligomers, proving that the kinase activity is not necessary for myddosome assembly. IRAK4 regulates myddosome formation through DD associations and serves as a kind of capping molecule which limits the size of MyD88 oligomers and prevents its further polymerization. Furthermore, IRAK4 is only recruited to oligomers of certain length, thus preventing random signalosome activation [106]. The kinase activity of IRAK4 can induce autophosphorylation in its N-terminal loop (Ser8). This phosphorylation prevents IRAK4 association with MyD88 DD. When myddosome-bound IRAK4 undergoes autophosphorylation, the complex is destabilized which can lead to myddosome disassembly [107]. Indeed, it has been reported that IRAK4 kinase inhibition causes myddosome stabilization [108]. Hence, not only does IRAK4 set a threshold for signaling initiation, but also has a crucial role in limiting the duration of IL-1R/TLR activation.

The kinase activity of IRAK4 was initially thought to be necessary for NF-κB activation in the IL-1R/TLR pathway and subsequent cytokine production [78]. This was disproved later in several studies of kinase inactive IRAK4, which revealed a kinase-independent NF-κB and MAPK activation in both human and murine cells [108-110]. The activation of MAPK and NF-κB pathway in IRAK4 kinase inactive cells is then likely accounted for by MEKK3-dependent IKK and MAPK activation, in contrast to the classical TAK1-mediated IKK and MAPK phosphorylation. Additionally, deletion of MEKK3 in cells with inactivating kinase dead IRAK4 (IRAK4 KD) mutation completely abolished NF-κB activation [111]. Complete deletion of IRAK4 in cells resulted in abolished NF-κB pathway after IL-1 stimulation [112]. IRAK4 is therefore necessary for the events upstream of NF-κB and MAPK, but its kinase activity in these pathways is dispensable, highlighting important scaffold function of IRAK4 in the myddosome. Interestingly, while the inactivation of IRAK4 kinase activity (genetic or chemical) appears to have little to no effect on NF-κB or MAPK activation, in some cells cytokine mRNA levels following stimulation are markedly reduced [108, 110]. Similarly, IRAK4 KD mice showed reduced levels of circulating cytokines after CpG or LPS challenge and are resistant to lethal septic shock [93].

The ablation of cytokine induction in certain cells after IRAK4 kinase inactivation might be explained by TLR downstream signaling through interferon regulatory factors (IRFs). This pathway has primarily been detected in plasmacytoid dendritic cells and appears characteristic of this cell type. In the context of TLR signaling, IRFs are a primary target of the MyD88-independent pathway (TLR3, TLR4), however, activation of these factors has been detected even following stimulation of MyD88 exclusive TLRs, namely TLR7, TLR8, and TLR9 [113]. IRFs are a family of transcription factors, which induce type I interferons, important in antiviral responses, as well as other inflammatory cytokines (e.g., IL-6, TNF). The MyD88-dependent pathway was reported to activate IRF5 and IRF7 [114, 115]. In cells treated with IRAK4 inhibitors, the nuclear translocation of IRF5 and IRF7 was drastically reduced. A proposed mechanism of IRF5/7 activation by IRAK4 is via the activation of TAK1 and IKKβ [116]. A possible explanation for the reduction of induced cytokine levels even with intact NF-κB and MAPK pathways may lie in the target gene regulatory sequences. Many cytokine genes have regulatory sequences with both NF-κB and IRF binding sites. It might be possible that in some cells cooperation between these transcription factors is necessary for effective gene transcription [117]. Additionally, it has been proposed that IRAK4 kinase activity may be able to influence the stability of cytokine mRNA [93]. To conclude, the kinase activity of IRAK4 is crucial for cytokine production in response to IL-1R/TLR stimuli.

Taken together, IRAK4 kinase-independent function is to provide a scaffold to which downstream molecules can associate and where they can interact to eventually ensure NF-κB and MAPK activation, while also inducing cytokine transcription and limiting the length of signalosome activation through its kinase activity.

4.2. IRAK4 deficiency

IRAK4 and MyD88 deficiency have a lot in common. Patients deficient in IRAK4, are susceptible to frequent infections with pyogenic bacteria (e.g., *S. pneumoniae*, *S. aureus*, *P. aeruginosa)* with high mortality at a young age with a steady decrease of mortality as patients age [37]. The mutations found in patients deficient in IRAK4 either create a premature stop codon or delete a substantial portion of the gene and are therefore predicted to completely prevent protein translation. Indeed, no IRAK4 protein levels were detected in cell lines derived from these patients. Furthermore, patient-derived peripheral blood mononuclear cells failed to induce appropriate

cytokine response when stimulated with TLR agonists (except for TLR3 agonist). Cytokine induction after TLR4 stimulation was impaired but not abolished which can be explained by TLR4 TRIF-dependent pathway activation [118].

Despite the life-threatening nature of IRAK4 deficiency, with early identification, patients can live a long life without any dire complications. Treatment with prophylactic antibiotics is recommended in IRAK4 deficient patients at least until the age of ten. This ought to decrease the incidence of invasive infections before proper adaptive immune response can be mounted. Additional incidental subcutaneous injections of IgG have also been proposed, since almost a third of the patients has impaired specific IgG antibody response. Finally, immediate antibiotic treatment should be administered when an infection is suspected, as time is of the essence and early treatment can prevent lethal septic shock [37].

5. IRAK4 as a therapeutic target

As a fundamental molecule in IL-1R/TLR signaling, IRAK4 alterations are often the cause of IL-R/TLR-mediated autoimmunities. The involvement of these receptor pathways in disease pathology was discussed earlier in the text. Here, the focus will be on the effects of IRAK4 in previously mentioned autoimmune disorders and how this knowledge can be used in the development of therapeutics.

5.1. IRAK4 in autoimmune diseases

Despite the uncertainties about the role of IRAK4 kinase activity in downstream signaling, it has been demonstrated that inactivating mutations in the kinase domain protect mice in several autoimmune disease models.

In murine experimental models of RA, IRAK4 KD mutation rescued mice from developing severe joint inflammation and bone destruction. Immune cell infiltration to the synovium was also markedly diminished, while T cell and B cell responses remained unaffected [119]. TLR7 stimulation of RA patient-derived cells treated with IRAK4 inhibitor led to decreased cytokine production and cell migration. Interestingly, IRAK4 inhibition reversed Th1 and Th17 polarization which otherwise contributes to disease severity [39].

In an imiquimod-induced murine model of psoriasis, IRAK4 inhibition resulted in a dose dependent improvement of the clinical features, most apparent in the reduction of skin scaling and erythema [120]. Recently, the mechanism of action of an approved pharmaceutic long used to treat psoriasis and multiple sclerosis was elucidated where IRAK4 and its interaction with MyD88 were identified as the main cellular target [121].

IRAK4 KD in mice with TLR7 overexpression model of SLE improved in all autoimmune markers associated with SLE, such as leukocyte activation, kidney immune cell infiltration, glomerulonephritis, or splenomegaly. IRAK4 kinase activity is therefore necessary for the development of autoimmune phenotype of SLE [122].

In MOG peptide induced EAE (murine model of MS), IRAK4 KD mice had a significantly lower incidence of EAE compared to WT. IRAK4 KD mice also showed decreased Th17 activation, measured as a reduction in Th17 cytokines. IRAK4 kinase activity is important in Th17

polarization, while its effect on Th1 and Th2 are minimal [123]. Since Th17 are the main driver in a range of autoimmune diseases, targeting IRAK4 is likely to alleviate the pathologic phenotype.

5.2. IRAK4 in cancer

The involvement of IRAK4 in tumorigenesis remains elusive. Signaling through IL-1 family receptors was shown to have mostly pro-tumorigenic effect, while the pro- or anti-cancer effects of TLRs largely depend on the type of cancer [74]. IRAK4 represents a potentially highly effective and cell-type specific cancer therapy target.

IRAK4 is implicated mainly in immune cell malignancies, though there is convincing evidence for IRAK4 in melanoma and colorectal cancer. An oncogenic mutation of MyD88 (L265P) in its TIR domain has been linked to activated B cell-like diffuse large B cell lymphoma. This mutation causes increased recruitment of IRAK4 and overactivation of IRAK1. IRAK4 KD did not affect cancer survival, while IRAK4 KO or combined inhibition of IRAK1 and IRAK4 was toxic to cancer cells [124]. MyD88 mutations are also present in another type of leukemia, chronic lymphocytic leukemia. Once again, these mutations cause overactivation of MyD88 which leads to increased signaling and cytokine production, resulting in a pro-tumorigenic microenvironment. Chemical inhibition of IRAK4 caused a decrease in cancer cell viability and proliferation [125]. In acute myeloid leukemia, an isoform of IRAK4 (IRAK4-L) generated by alternative splicing is induced. This isoform leads to increased phosphorylation and activation of IRAK1, p38, JNK, and NF-κB in comparison to WT form. IRAK4-L is necessary for leukemic cell function as knockdown of IRAK4-L reduced leukemic burden [126].

Melanoma cells display high levels of phosphorylated IRAK4 in resting state, suggesting constitutive IRAK4 activation, but the phosphorylation could still be increased with TLR stimulation. IRAK4 inhibition resulted, among others, in reduction in phosphorylated NF-κB and decreased production of vascular endothelial growth factor. *In vitro* studies of several melanoma cell lines discovered IRAK4 inhibition can sensitize them to chemotherapy even if the effects of chemotherapy alone are minimal [127]. Analogous findings have been made in pancreatic ductal adenocarcinoma (PDAC), where IL-1β produced by stromal cells leads to constitutive IRAK4 activation in cancer cells. IRAK4 inhibition or genetic ablation resulted in reduced growth of PDAC and increased chemotherapy toxicity in PDAC cells [128]. In colorectal cancer, chemotherapy can lead to IRAK4 and NF-κB overactivation. This in turn may contribute to the

development of chemotherapy resistance. Administration of IRAK4 inhibitor reverses these effects and works in synergy with chemotherapy to promote cancer cell apoptosis [129]. A recent study of breast cancer indicated IRAK4 has negative effects on tumor growth and its suppression led to faster growth and proliferation [130]. This is in contradiction to findings in other malignancies, underlining the need for detailed diagnosis when targeting IRAK4 in cancer.

6. Drugs targeting IRAK4

IRAK4 is a rational target for therapy design since it is involved in many autoimmune diseases and some types of cancer. Several approaches to IRAK4 inhibition can be taken. Firstly, the kinase activity can be targeted, resulting in downstream signaling attenuation. This however does not account for the IRAK4 kinase-independent pathway. Other tactic can be to aim at IRAK4 interaction with other molecules, most notably the DD interaction with MyD88. Finally, if complete ablation of IRAK4 is the goal, it is possible to employ either RNA interference, which is costly and complicated, or targeted protein degradation.

In this chapter, chemical inhibitors and protein degraders will be discussed and where available, preliminary data from clinical trials will also be examined.

6.1. Chemical inhibitors

There is a plethora of small molecule inhibitors of IRAK4 that have been and are being developed. No IRAK4 inhibitors have yet reached use in clinic, however many are now undergoing testing at different stages of clinical trials. Bellow, inhibitors that are currently in clinical trials are described.

R289 (developed by Rigel Pharmaceuticals) is an orally administered combined inhibitor of IRAK1 and IRAK4. Its administration provides reduction of inflammatory cytokine production. In mouse models of arthritis, serum cytokine levels were decreased. In a rat model of collagen-induced arthritis, treatment with R835 (active metabolite of R289) reduced cartilage degradation and synovial inflammation [131]. A clinical trial evaluating drug safety in patients with myelodysplastic syndromes is now in preparation (trial ID NCT05308264).

Zabedosertib (BAY 1834845, developed by Bayer) is an IRAK4 inhibitor that was most extensively tested on murine model of SLE. This inhibitor was proved to work selectively in the MyD88-dependent pathway and the data obtained from mice treated with inhibitor were comparable to IRAK4 KD mice. Most MyD88-dependent responses were also inhibited in human peripheral blood mononuclear cells derived from lupus patients [132]. Phase I of clinical trial on healthy males or male patients with psoriasis has been completed (trial ID NCT03493269), however the results have not been made public yet. Another trial is underway, focusing on safety in healthy males (trial ID NCT05003089).

Zimlovisertib (PF-06650833, developed by Pfizer) is a highly potent IRAK4 inhibitor intended for the treatment of RA and SLE. In mouse model of SLE, zimlovisertib reduced kidney inflammation. In phase I clinical trials on healthy individuals, the inhibitor reduced levels of C-reactive protein and type I IFN, which are usually increased in SLE, demonstrating its potency to reduce markers of inflammation in humans [133]. Currently, it is also being tested for its effects on the inflammatory state in patients with severe COVID-19 (trial ID NCT04933799).

Emavusertib (CA-4948, developed by Curis, Inc.) aims to target B cell malignancies with IRAK4 involvement, as well as acute myeloid leukemia and myelodysplastic syndrome [134]. Recently a partial hold was placed on phase II clinical study of emavusertib alone or in combination with azacitidine or venetoclax (standard chemotherapy for leukemia) due to a participant's death with condition resembling dose-limiting toxicity of emavusertib (trial ID NCT04278768). Another clinical trial focuses on emavusertib and combined treatment with ibrutinib (commonly used to treat B cell cancers) in relapsed hematologic malignancies (trial ID NCT03328078).

HS-243 is an inhibitor of IRAK1 and IRAK4 developed in an independent study. It shows promising data on its potential use in RA, as well as in pancreatic, ovarian or colorectal cancer [135]. As of date, HS-243 has not entered clinical trials.

AZ1495 (Astra Zeneca) is an inhibitor of IRAK4 which seems to be active in the treatment of MyD88 L265P mutation driven B cell lymphoma [136]. AZ1495 is currently not being tested in clinical trials.

6.2. PROTAC inhibitors

An emerging approach in the field of highly specific therapeutics are proteolysis targeting chimeras (PROTACs). These molecules are currently an attractive field of study since they provide an opportunity to aim at proteins that have thus far been difficult to target with conventional drug design. Nowadays, PROTACs are heterobifunctional small molecules with three distinguishable parts: targeting moiety region which binds to the protein of interest, linker, and an E3 handle for binding of a ubiquitin ligase (Fig. 7). The goal of a PROTAC is to bring ubiquitin ligase to close proximity with the target protein and catalyze addition of K48-Ub to that protein. Once marked with K48-Ub, targeted protein is degraded by the proteasome (Fig. 8). The range of E3 Ub ligases utilized by PROTACs is limited at the moment. Amongst the most commonly used are inhibitors of apoptosis (IAPs), murine double minute 2 (MDM2), and cereblon (CRBN) [137]. Adding more

E3 ligases to the repertoire might be a useful direction of future PROTAC design. Targeted degradation is especially useful in proteins whose inhibition is not sufficient for the nullification of its adverse effects. PROTACs are usually administered orally or intravenously [138].

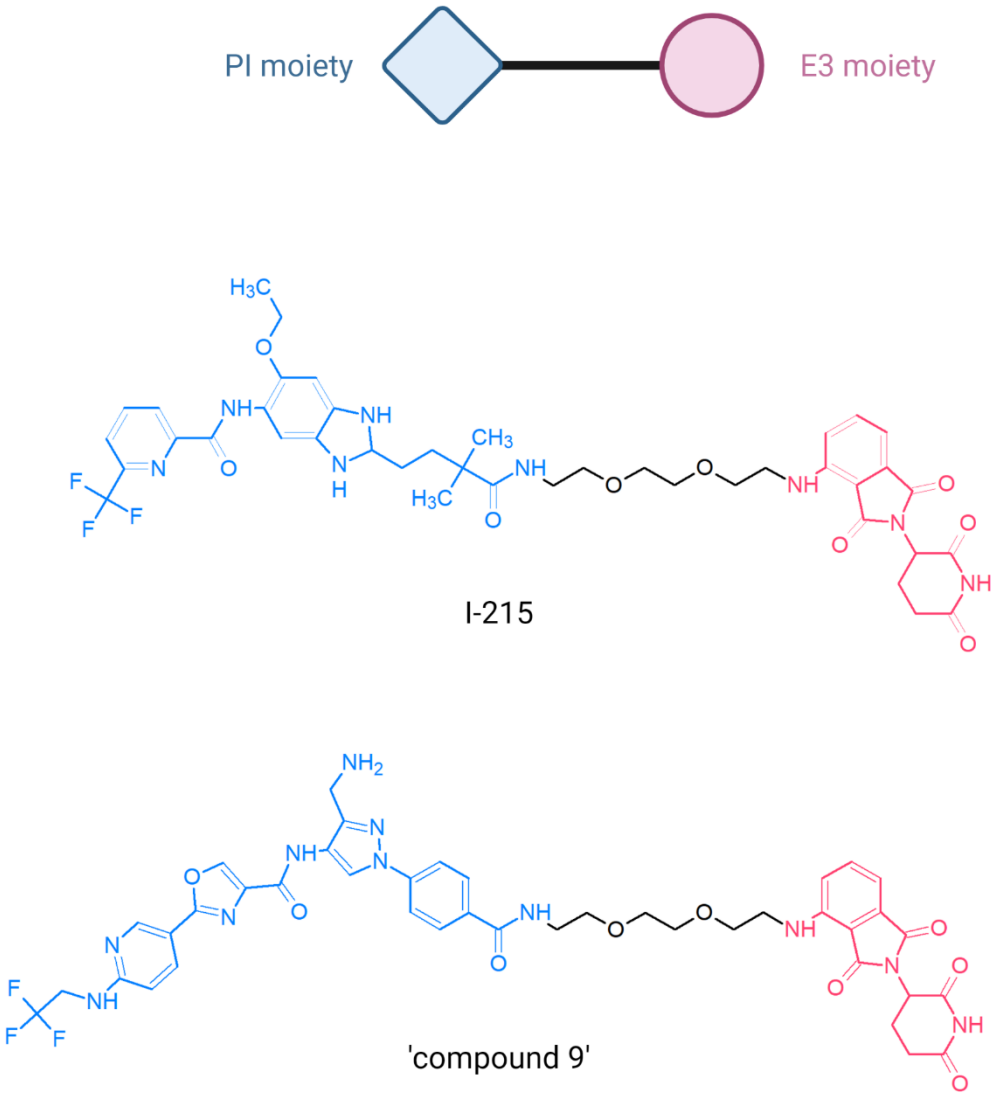

Figure 7. General structure of PROTAC degraders with two examples: *Prototypic PROTAC contains protein of interest moiety for binding to the targeted protein, linker of variable length, and ubiquitin ligase moiety for binding to the ubiquitin ligase. I-215 is one of several compounds patented by Kymera Therapeutics [139]. Compound 9 is a PROTAC developed as a part of a study comparing different linker lengths [140]. Created with BioRender.com*

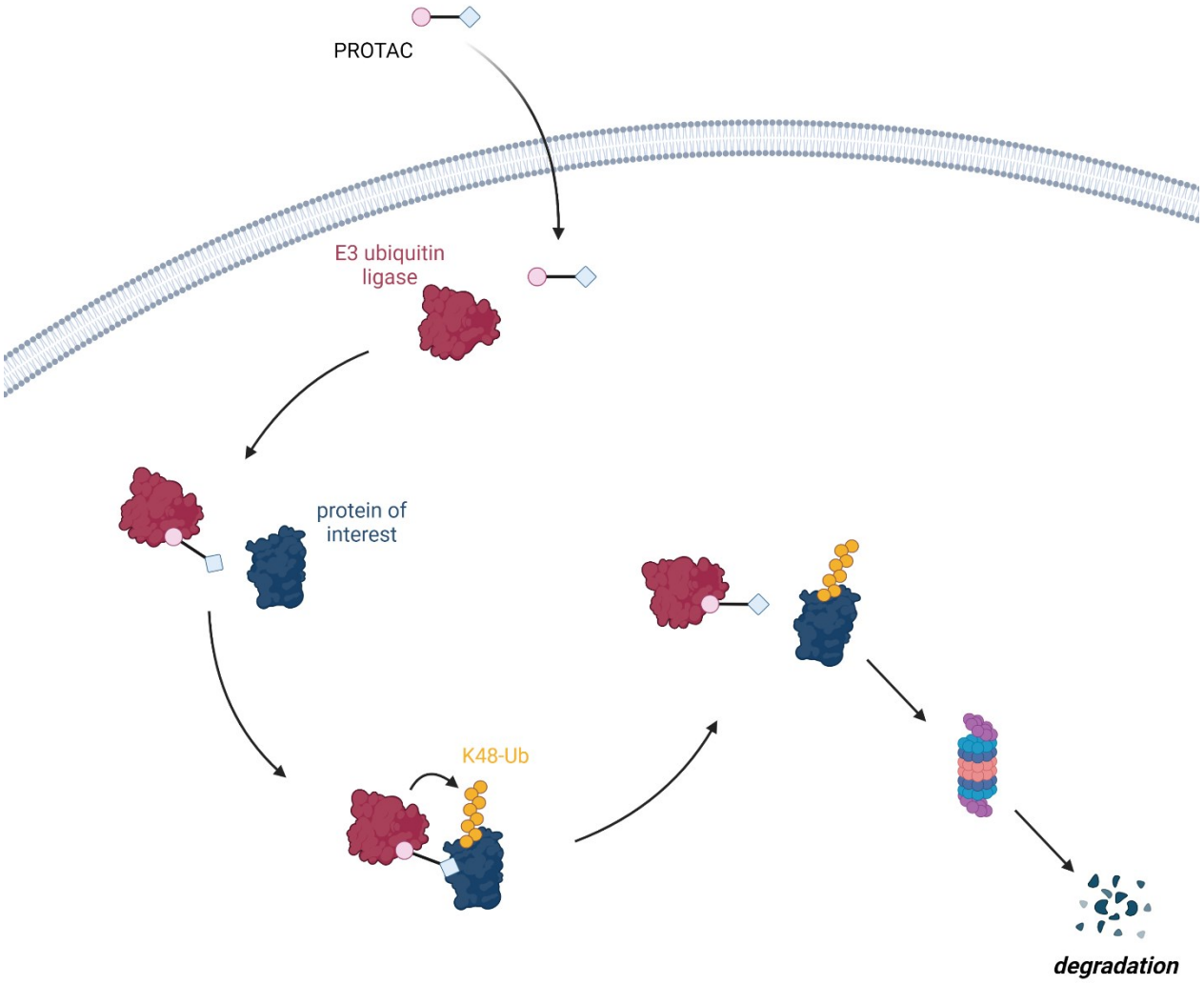

Figure 8. PROTAC mechanism of action: *PROTAC binds to ubiquitin ligase and protein of interest with its respective moieties. Protein of interest is then marked with K48-polyubiquitin and subsequently degraded by the proteasome. Created with BioRender.com*

Since IRAK4 acts in two ways in the myddosome, as a kinase and as a scaffold, PROTACs provide a great opportunity for IRAK4-mediated disease treatment. The fact that IRAK4 is a cytoplasmic protein, has known binding sites for small molecules, and can be polyubiquitinated makes it a perfect candidate for PROTAC therapy. Indeed, several IRAK4 degraders have been developed and two are now in clinical trials.

KT-474 (Kymera Therapeutics/Sanofi) is now the lead IRAK4 protein degrader. It showed high efficacy in preclinical studies and is the first IRAK4 targeting PROTAC to enter clinical trials. It is currently in phase I of testing with the goal to determine safety and outcomes of multiple ascending doses on healthy individuals and patients suffering from atopic dermatitis or hidradenitis suppurativa (trial ID NCT04772885). Kymera did not disclose the PROTAC structure or employed E3 ligase.

KT-413 (Kymera Therapeutics) is a degrader with ambitious aim to target IRAK4 along with Ikaros and Aiolos, transcription factors regulating B and T cell development, in the treatment of MyD88 mutant diffuse large B cell lymphoma. Patients with relapsed or refractory B cell Non-Hodgkin lymphoma and healthy individuals are now being recruited to a trial to determine safety and pharmacokinetics of KT-413 (trial ID NCT05233033).

'Compound 9' is a degrader developed in a study of IRAK4 PROTACs with a potent IRAK4 inhibitor, CRBN moiety, and linker of varying size. Compound 9 with a PEG2 linker displayed much more efficient protein degradation compared to compounds with shorter linker. When tested on lymphoma cell lines (OCI-LY10, TMD8), compound 9 had a significant antiproliferative effect. This effect was higher than when cells were treated with IRAK4 inhibitor alone. Additionally, compound 9 targeted MyD88 mutant lymphoma cells with high selectivity in comparison to WT MyD88 [140].

Altogether, the data obtained from *in vitro* studies and clinical trials demonstrate that IRAK4 is a good target in a wide variety of diseases. However, cell specific action of IRAK4 needs to be further inspected and taken into account when choosing IRAK4-based treatment.

7. Conclusion

Signaling through Toll-like and IL-1 family receptors is a vital component of the immune system. They recognize various invading pathogens as well as self-components indicative of cell damage, all to prevent infection and maintain immune homeostasis. Though classified mostly as a part of innate immunity, these receptors also play an important role in the development of adaptive immunity. Dysregulation of the IL-1R/TLR pathway therefore has broad systemic effects and can lead to development of autoimmune diseases or cancer.

MyD88 and IRAK4, shared by both receptor families, are the first to interact with the receptor following stimulation. Their importance is demonstrated by increased, life threatening susceptibility to infection and sepsis in their absence. On the other hand, overactivation can cause sustained systemic inflammation through the activation of the NF-κB and MAPK pathways and drive diseases such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis and promote tumor microenvironment. This makes MyD88 and IRAK4 attractive targets for therapy. Since MyD88 is an adaptor protein and would be hard to effectively inhibit, IRAK4 is at the center of attention in the development of pharmaceutics in this pathway. Indeed, countless inhibitors are being made with many advancing into the final stages of clinical testing. The available information from pre-clinical trials and preliminary data from the first stages of clinical trials are very promising so far. Since IRAK4 poses both as a kinase and adaptor, it is also a good candidate for treatment with compounds form the growing field of targeted protein degradation. IRAK4 protein degraders seem to be potentially very effective in the treatment of hematologic malignancies with high selectivity for cancer cells.

Despite the advancements made in understanding of MyD88, IRAK4 and their downstream effects, a lot remains a mystery. In future research, focusing on the cell specific activities of these proteins might provide necessary information that could help create more personalized and highly effective therapies.

8. References

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