OPEN

IRAK1 and IRAK4 as emerging therapeutic targets in hematologic malignancies

Joshua Bennett[a,b] *and Daniel T. Starczynowski*[a,b,c]

Purpose of review
Cell intrinsic and extrinsic perturbations to inflammatory signaling pathways are a hallmark of development and progression of hematologic malignancies. The interleukin 1 receptor-associated kinases (IRAKs) are a family of related signaling intermediates (IRAK1, IRAK2, IRAK3, IRAK4) that operate at the nexus of multiple inflammatory pathways implicated in the hematologic malignancies. In this review, we explicate the oncogenic role of these kinases and review recent therapeutic advances in the dawning era of IRAK-targeted therapy.

Recent findings
Emerging evidence places IRAK signaling at the confluence of adaptive resistance and oncogenesis in the hematologic malignancies and solid tissue tumors. Preclinical investigations nominate the IRAK kinases as targetable molecular dependencies in diverse cancers.

Summary
IRAK-targeted therapies that have matriculated to early phase trials are yielding promising preliminary results. However, studies of IRAK kinase signaling continue to defy conventional signaling models and raise questions as to the design of optimal treatment strategies. Efforts to refine IRAK signaling mechanisms in the malignant context will inspire deliberate IRAK-targeted drug development and informed combination therapy.

Keywords
acute myeloid leukemia, adaptive resistance, hematologic malignancies, interleukin 1 receptor-associated kinase inhibitors, interleukin 1 receptor-associated kinases, myelodysplastic syndromes, signaling mechanisms

INTRODUCTION

The vertebrate innate immune system encompasses a network of phylogenetically conserved molecular and cellular mechanisms that have evolved as an inborn defense against pathogens. A defining feature of the innate immune system, conducted by the toll-like receptors (TLRs), is the ability to recognize generic molecular patterns associated with bacteria, fungi, viruses, and cellular debris. The TLRs, with minor exceptions, converge on the interleukin 1 receptor-associated kinases (IRAK1, IRAK2, IRAK3, IRAK4) to coordinate multiple inflammatory pathways involved in cell survival, cytokine production, and priming of the adaptive immune system. Several recent reviews have meticulously outlined genetic and molecular evidence that cooptation of IRAK-signaling pathways is intrinsic to the pathobiology of the hematologic malignancies [1–9]. These observations have ignited interest in the IRAK kinases as therapeutic targets and thereby renewed focus on resolving IRAK-signaling mechanisms. Accumulating evidence indicates that IRAK signaling is more dynamic and member-specific than what is ascribed by simplistic conventional signaling models. These emerging idiosyncrasies have critical implications for how best to manipulate the IRAK kinases to subvert aberrant inflammatory signaling in malignancy. Below, we provide a contemporary overview of the IRAK kinases in the context of healthy and malignant biology. We profile IRAK-targeted therapies and enumerate essential research mandates for the field.

[a]Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, [b]Department of Cancer Biology and [c]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

Correspondence to Daniel T. Starczynowski, Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA. Tel: +1 513 803 5317; e-mail: Daniel.Starczynowski@cchmc.org

Curr Opin Hematol 2022, 29:8–19

DOI:10.1097/MOH.0000000000000693

KEY POINTS

- The IRAKs support oncogenic signaling pathways and chemoresistance in diverse malignancies.
- Early clinical trials validate the IRAK kinases as tractable drug targets.
- IRAK signaling redundancies and compensation mechanisms are uncharacterized.
- IRAK2 and IRAK3 are understudied as oncogenic effectors.

CONVENTIONAL SIGNALING PARADIGM OF INTERLEUKIN 1 RECEPTOR-ASSOCIATED KINASES

IRAK4

IRAK4 is a 460 amino acid threonine/serine kinase and the most recent IRAK family member to be identified [10]. Upon ligation, the TLRs and IL-1 Receptor complex (IL-1R1 and IL-1RAcP) dimers undergo a conformational shift that re-orients their cytoplasmic TIR domains [11,12]. Heterotypic IL1R/IL1RAcP and homotypic TLR TIR-TIR dimers (except in the case of TLR3) subsequently recruit the TIR domain-containing adaptor molecule MyD88 [13]. The N-terminal death domains of MyD88, IRAK4, and IRAK2 then facilitate the assembly of a multimeric, helical signaling complex (Myddosome) consisting of six molecules of MyD88, four molecules of IRAK4, and four molecules of IRAK2 [14,15]. IRAK4 dimers recruited to the Myddosome undergo asymmetric trans-autophosphorylation [16] and recruit IRAK1, which then itself participates in extensive autophosphorylation and dissociates from the Myddosome (Fig. 1) [17]. Intriguingly, the absolute requirement of IRAK4 kinase activity for canonical signaling is not firmly established, and few IRAK4 substrates have been characterized. Vollmer *et al.* [18] found that chemical inhibition of IRAK4 autophosphorylation in IL-1R cells had a negligible effect on IRAK1 activity and NF-κB/P38/JNK signaling upon IL-1 stimulation. Others have similarly demonstrated that expression of kinase-dead IRAK4 isoforms in IRAK4-deficient cells fully restores IRAK1 phosphorylation and signaling downstream of IL-1 [19,20]. In an IL-1-stimulated fibroblast model, Ferrao *et al.* [16] found that ablation of catalytic activity or dimerization only moderately impaired the ability of IRAK isoforms to conduct signaling and induce downstream cytokine expression. Conversely, Wang *et al.* [21] found that only the phosphorylated species of IRAK4 was capable of heterodimerizing with IRAK1. In the human monocytic acute myeloid leukemia (AML) THP-1 cell line, an IRAK4 kinase inhibitor demonstrated an equivalent ability to impair TLR4-induced signaling through the NF-κB, JNK, and MAPK pathways as an IRAK4 PROTAC built on the same compound, suggesting that IRAK4 kinase activity does, in fact, mediate signaling [22]. However, both the IRAK4 inhibitor and proteolysis-targeting chimera (PROTAC) degrader only modestly attenuated canonical signaling, calling into question whether Myddosome assembly entirely requires IRAK4 in any capacity [22]. Collectively, though some studies report a requisite role of IRAK4 catalytic activity for signal transduction and cytokine induction in human myeloid cells [10,23,24], the majority of experimental evidence insinuating an absolute requirement of IRAK4 catalytic activity in canonical signaling comes from mouse models. This is consistent with the published finding that murine myeloid cells are far more dependent on IRAK4 for inflammatory signaling than human myeloid cells [25]. Thus, the requirement of IRAK4 kinase activity may vary by cell type, upstream stimulus, and pathological context.

IRAK1

IRAK1 is a threonine/serine kinase. Unlike IRAK4, IRAK1 is not a core constituent of the Myddosome but transiently interacts with IRAK4 upon Myddosome assembly. Unlike IRAK4, IRAK1 exists primarily in an inactive conformation at the basal state and requires upstream stimulation for rapid activation [18]. IRAK1 is reported to be a phosphorylation substrate of IRAK4 [10] and is subject to extensive ubiquitylation and autophosphorylation proceeding IL-1R/TLR stimulation [17,26]. However, IRAK4-mediated activation of IRAK1 kinase activity occurs by an allosteric mechanism rather than by covalent modification [18]. Once dissociated from the Myddosome, IRAK1 interacts with and activates the E3 ligase TRAF6 to induce canonical signaling through the NF-κB and MAPK pathways [27] (Fig. 1). Intriguingly, lysine (K) 63-linked polyubiquitylation of IRAK1 is necessary for TRAF6-mediated signal transduction, suggesting that covalent modification of IRAK1 may regulate its accessibility as a scaffolding substrate [17,28]. Precisely how IRAK1 stimulates TRAF6 remains obscure, though it is apparently independent of IRAK1 catalytic activity; thus, in at least some contexts, the canonical IRAK signaling axes are robust to the isolated loss of either IRAK1 or IRAK4 kinase activity. It is tempting to speculate that IRAK1 and IRAK4 maintain catalytic redundancy in this regard, therefore, requiring a bispecific approach to terminate canonical signaling by small molecule

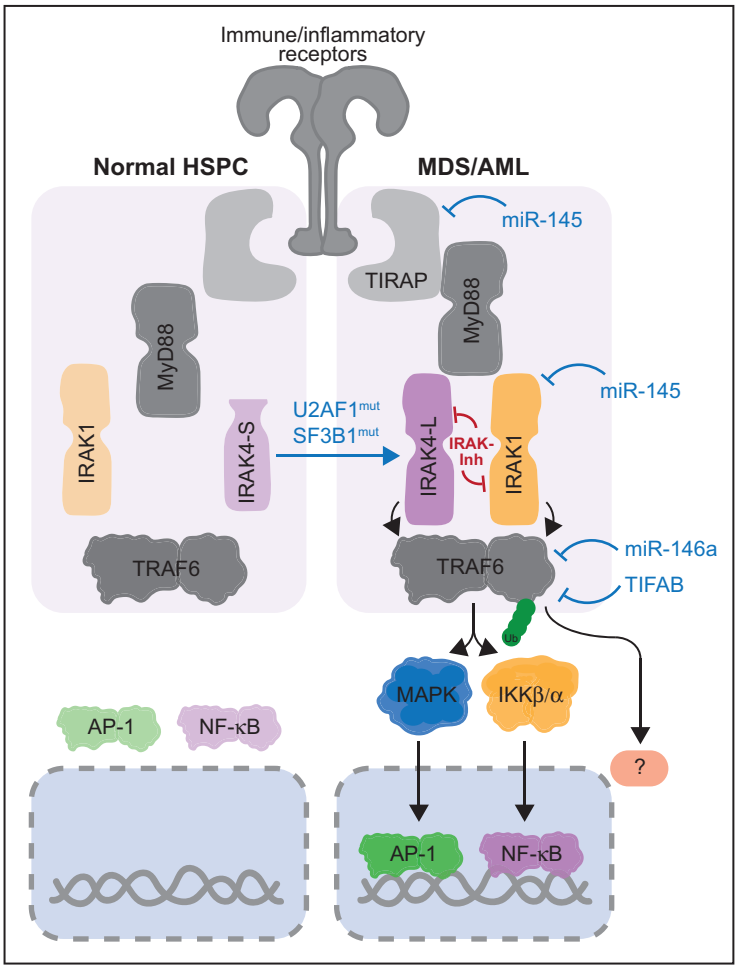

FIGURE 1. Dysregulation of interleukin 1 receptor-associated kinase-dependent signaling in myeloid malignancies. Inflammatory and immune-related receptors, such as certain TLRs and IL-1R, recruit the adaptors TIRAP and MyD88, along with IRAK kinases and TRAF6, to form the Myddosome complex. Negative regulators of the pathways, such as miR-145, miR-146a, and TIFAB, are frequently deleted in MDS and AML. Mutations in U2AF1 and SF3B1 result in conversion of hypomorphic (IRAK4-S) to hypermorphic (IRAK4-L) IRAK4 isoforms in MDS and AML. IRAK4-L can recruit MyD88 and IRAK1 to activate signaling to NF-κB and MAPKs. The signaling pathway was adapted from Trowbridge and Starczynowski (2021). AML, acute myeloid leukemia; IRAK, interleukin 1 receptor-associated kinases; MDS, myelodysplastic syndromes; TLRs, toll-like receptors.

inhibition. We discuss preliminary efforts to target the IRAK kinases by such an approach below.

Recent efforts have focused on the role of IRAK1 in activating NLRP3 inflammasome assembly. Several groups have reported that IRAK1 regulates rapid NLRP3 inflammasome assembly and caspase 1 cleavage following IL-1R/TLR ligation in a transcription-independent fashion [29,30]. Notably, and in contrast to Myddosome signaling via TRAF6, NLRP3 inflammasome assembly is dependent on the catalytic competency of both IRAK1 and IRAK4 [29]. These findings contradict to a study that asserts a role for IRAK1 as a repressor of NLRP3 inflammasome assembly [31]. The IRAK1 substrates and interactors in this context are not characterized, and temporally segregated interactions with distinct binding partners may modulate the influence of IRAK1 on inflammasome assembly.

IRAK2

IRAK2, like IRAK4, is a core constituent of the Myddosome complex. Unlike IRAK1 and IRAK4, IRAK2 is predicted to be a pseudokinase as it lacks a critical aspartate residue in the VIb kinase subdomain [32] and exhibits negligible kinase activity *in vitro* [33]. However, the fundamental signaling mechanisms by which IRAK2 operates have not been subject to the same level of scientific scrutiny as have IRAK1 and IRAK4, and evidence from human models is conspicuously absent. This is especially problematic as the utilization of IRAK2 in mice and humans is

discrepant [25]. As with IRAK1, IRAK2 contains TRAF6-binding sites [34], and IRAK2 overexpression is sufficient to induce TRAF6 ubiquitylation and NF-κB signaling in human cell lines [35,36]. Moreover, knockdown of IRAK2 in human cell lines suppresses NF-κB signaling in response to TLR stimulation [36]. Several investigations in mouse models posit that IRAK2 is indeed catalytically active and maintains late-phase NF-κB signaling and sustained cytokine production downstream of TLR stimulation [37–40]. IRAK2 has not been convincingly demonstrated to possess such catalytic competence and latent signaling regulation in human biology.

IRAK3

IRAK3, also known as IRAK-M to reflect its predominant expression in monocytes, is also a pseudokinase [33]. In contrast to the other IRAK family members, studies in human models indicate IRAK3 is a negative regulator of Myddosome signaling [41–44], which supports the association of inactivating IRAK-M mutations with early-onset asthma [45]. Like all family members, IRAK3 possesses an N-terminal death domain, and as with IRAK1 and IRAK2, a C-terminal domain with TRAF6 binding sites [34]. IRAK3 is, thus equipped to engage the Myddosome complex and modulate TRAF6, though the exact mechanism by which IRAK3 suppresses Myddosome signaling is obscure. Crystal structure analysis of the IRAK3 pseudokinase domain predicts a higher-order assembly of IRAK3 dimers and IRAK4 that would occlude the IRAK4 catalytic site, presenting a plausible means of mitigating Myddosome signaling [46]. Along these lines, mutagenesis of N-terminal IRAK3 residues predicted to mediate interaction with IRAK4 abrogate the ability of IRAK3 to dampen TLR signaling [42]. These interactions with IRAK4 may serve to prevent Myddosome disassembly and TRAF6 activation, as observed previously [41]. IRAK3 also possesses intrinsic guanylate cyclase activity central to its regulatory competence, possibly by creating a localized cGMP gradient that stabilizes the Myddosome [43]. Finally, an alternative model posits that an IRAK3-integrated Myddosome complex engages a MEKK3-dependent NF-κB pathway that drives the expression of anti-inflammatory genes [47].

ROLE OF INTERLEUKIN 1 RECEPTOR-ASSOCIATED KINASES IN HEMATOLOGIC MALIGNANCIES

Inflammatory dysregulation is a fundamental feature of pathogenesis in the hematologic malignancies. Multiple oncogenic signaling nodes converge on the IRAK kinases, generating significant interest in these kinases as targetable effectors of malignancy. In this section, we discuss accumulated evidence pertaining to the role of the IRAK kinases in the hematologic malignancies.

Myelodysplastic syndromes

The myelodysplastic syndromes (MDS) are a group of diseases characterized by ineffective hematopoiesis and myelodysplasia driven by the clonal dominance of defective hematopoietic stem cells (HSC) [48]. Many of the genetic lesions associated with MDS induce cell-intrinsic alterations in the IRAK-signaling hub [49–54]. One of the most common subtypes of MDS, 5q-syndrome, is mediated by the deletion of microRNAs (miR-145 and miR-146a) and regulatory proteins (TIFAB) that target IRAK1 and TRAF6 (Fig. 1). Haploinsufficiency of these genes depresses TRAF6 and IRAK1 and promotes NF-κB-dependent myeloproliferation [51,52,55–58]. TLR1, TLR2, TLR4, TLR6, MYD88, and IRAK1 are frequently overexpressed in MDS HSC, supporting a functional role for IRAK signaling in MDS pathobiology [59–63]. Rhyasen *et al.* [64] reported that inhibition of IRAK1 reduced progenitor function and viability in MDS HSC but not healthy $CD34^+$ bone marrow cells. Driver mutations in the splicing factors U2AF1 and SF3B1 that induce hypermorphic IRAK4 isoforms are recurrent in MDS and AML and impose sensitivity to IRAK4 inhibition [65,66]. Stromal elaboration of the alarmins S100A8 and S100A9, which ligate TLR4, drives genotoxic stress and preleukemic evolution, suggesting the IRAK-signaling axis may play a pivotal role in the emergence of MDS HSC [67]. Indeed, the alarmins are noted to be elevated in the plasma of low-risk and intermediate-risk MDS [68] and proposed to serve a multifaceted role in disease progression by enforcing an erythroid differentiation block, driving myeloid-derived suppressor cell (MDSC) expansion, and instigating NRLP3 inflammasome activation [50,69–71,72▪]. S100A9 binding to the CD33 complex on MDSC promotes MDSC outgrowth and the production of cytokines that suppress hematopoiesis and antitumor immunity [71] MDSC are overrepresented in the bone marrow of MDS, and their abundance is a negative prognostic factor [73]. MDSCs themselves produce S100A8/9, instituting a forward TLR signaling feedback loop [74]. The S100A8/9 alarmins also enforce hematopoietic dysfunction in MDS by triggering NLRP3 inflammasome assembly and pyroptotic cell death in MDS HSCs via TLR4 signaling [72▪]. As discussed above, IRAK1 is a critical mediator of NLRP3 inflammasome assembly downstream of the TLRs [29,30].

Inflammasome priming also requires TLR-IRAK-mediated activation of NF-κB to induce NRLP3 and IL-1β expression [75]. IRAK1 thereby plays a dual role in regulating the NLRP3 inflammasome at the transcriptional and protein level. Furthermore, the NLRP3 inflammasome facilitates the maturation and release of IL-1 β, a cytokine that signals through the IRAK kinases. Overexpression of IL1RAcP is another common feature in MDS HSC, and IL-1 signaling drives the selective expansion of malignant myeloid clones [76]. This is consistent with the recent finding that chronic exposure to the TLR ligand lipopolysaccharide (LPS) elicits a competitive advantage of MDS HSCs in chimeric bone marrow mouse models [77]. Collectively, these findings suggest that IRAK signaling downstream of the IL-1 receptor or TLR family favors the dominance of MDS clones. Thus, the IRAK signaling complex resides at multiple nodes in a complex inflammatory circuit governing MDS and represents a logical target to subvert various disease mechanisms concurrently.

Acute myeloid leukemia

MDS often antecedes AML, and many of the IRAK-signaling dependencies in MDS are preserved in AML. In an induced pluripotent stem cell (iPSC) model that harnessed sequential CRISPR/Cas9 mutagenesis to recapitulate clonal progression to AML, Wang *et al.* integrated transcriptional and epigenetic analyses to consolidate genetic dependencies in the transformation to overt leukemia. These dependent genes were heavily enriched for inflammatory mediators, and an IRAK1/IRAK4 dual inhibitor subverted progenitor function in leukemic, but not parental, iPSC-derived hematopoietic cells [78▪]. These findings suggest that signaling through the IRAK kinases is recruited early in the transition to AML. Mutually exclusive expression of a hypermorphic long IRAK4 (IRAK4-L) isoform over a hypomorphic short (IRAK4-S) isoform predicts clinical aggressiveness and worse outcomes in AML patients [65]. Treatment with an IRAK4 inhibitor potently suppresses leukemic function in AML cell lines with predominant IRAK4-L expression [65]. In concordance, parallel inhibition of the JNK and NF-κB pathways, which are both regulated by the IRAK kinases, effectively eradicates AML stem and progenitor cells [79]. As mentioned previously, IL-1 signaling favors the competitive advantage of leukemic clones in MDS and AML [76]. IRAK1 hyperphosphorylation is also observed in AML of heterogeneous genetic backgrounds, and IRAK1 inhibition reduces viability and in vivo leukemic burden across diverse AML cell lines and primary patient samples [80]. Nevertheless, the precise role of IRAK1/4 signaling dependencies in AML remains unknown. Whether the efficacy of IRAK1 inhibition and IRAK4 inhibition is mediated by repression of mutual or divergent signaling pathways, or, in the latter case, whether functional redundancy permits reciprocal compensation is not clear. These questions are of considerable clinical interest, as the attainable development of dual selective IRAK1/4 inhibitors may offer substantial therapeutic benefit over extant compounds. Furthermore, the requirement of upstream receptor ligation to prime the oncogenic function of the IRAK kinases in AML is obscure. These questions must be a priority for future investigations.

Other leukemias

IRAK4 has emerged as a target in chronic lymphocytic leukemia, which occasionally harbors driver mutations in MYD88 and TLR receptors [81–83]. MYD88-mutated CLL cases manifest an NF-κB signature, though treatment with an experimental IRAK4 inhibitor reduces viability and proliferation in patient-derived CLL regardless of MYD88 status [84,85]. IRAK1 is overexpressed in multiple T-ALL subtypes, which undergo apoptosis and cell-cycle disruption upon IRAK1 knockdown [86]. Notably, treatment with an IRAK inhibitor in this study only partially recapitulated the efficacy of protein knockdown, hinting that kinase-independent IRAK functions are indeed relevant to these malignancies [86]. A polymorphism that is predicted to mitigate the ability of miR146a – one of the microRNAs deleted in del(5q) MDS – to repress IRAK1 and TRAF6 is associated with an increased incidence of childhood ALL in a Taiwanese cohort [87]. TLR activation is observed in lymph node-resident CLL cells, and combination therapy of an IRAK1/4 inhibitor with the BTK inhibitor Ibrutinib demonstrated superiority over single-agent therapy in a preclinical study [88]. IRAK3, a suppressor of the IRAK1/2/4-signaling complex, is robustly expressed in monocytes exposed to circulating leukemic cells in CML patients via engagement of TLR4 and CD44 [89]. IRAK3 induction is theorized to deactivate monocytes and enable immune tolerance in CML [89]. Therefore, targeting IRAK3 in myeloid cells may facilitate an antitumor immune response in CML and possibly other heme malignancies.

Lymphomas

Activating MYD88 mutations that promote tonic signaling through the MyD88-IRAK signaling complex are found in nearly all cases of Waldenstrom's

macroglobulinemia and in a substantial portion of the Activated B-cell (ABC) subtype of Diffuse Large B-Cell Lymphoma (DLBCL) [90–92]. Primary lymphoplasmacytic cells collected from the bone marrow of MYD88-mutant Waldenstrom's macroglobulinemia patients on sustained ibrutinib therapy demonstrate elevated IRAK1 and IRAK4 phosphorylation and synergistic sensitivity to combination therapy of ibrutinib with an IRAK1/4 inhibitor *in vitro* [93]. An RNAi screen and treatment with an IRAK1/4 inhibitor identified both IRAK1 and IRAK4 as molecular dependencies in MYD88-mutant ABC DLBCL [92]. Recently, Hatcher *et al.* [94] discovered a highly selective inhibitor of IRAK1 that displays antiproliferative potency in MYD88 mutant lymphoma.

Likewise, a Pyrrolopyrimidine compound with optimized IRAK4 selectively has been developed and found to reduce NF-κB signaling and MYD88-mutant lymphoma viability [95]. It is worth noting that several studies in MYD88 mutant lymphomas describe a predominant reliance on IRAK1 over IRAK4 [22,93,96], and genome-wide CRISPR screens have implicated IRAK1, but not IRAK4, as a significant molecular dependency in ABC DLBCL [97,98]. Indeed, one study reported that IRAK4 was entirely dispensable to ABC DLBCL survival [22]. These data challenge the dogma that IRAK1 absolutely requires upstream recruitment by IRAK4 to initiate signaling, at least in the context of malignant biology. Rather, IRAK4 and IRAK2 may be interchangeable in the mutant Myddosome, or IRAK1 may assert additional Myddosome-independent oncogenic function. Such possibilities should be explored in future studies. Given the equivocal requirement of IRAK kinase activity in Myddosome signaling as alluded to previously, it is reasonable to question whether ATP-competitive small molecule inhibitors are ideal modalities for IRAK-directed therapy. In fact, the studies demonstrating the utility of IRAK1/4 inhibitors in MYD88 mutant lymphoma only observed efficacy in the micromolar ranges, far above the respectively reported IC_{50} values [91,92,94,95]. In one study of ABC DLBCL, kinase-dead IRAK1 was able to rescue viability following IRAK1 knockdown [92]. Conversely, a recently generated series of IRAK1 PROTACS exhibited antiproliferative potency against ABC DLBCL in the low nanomolar range [99▪]. These findings indicate the scaffolding functions of IRAK1 facilitate the oncogenic signaling in MYD88-mutant ABC DLBCL.

The IRAK kinases are also implicated in other B-cell malignancies. Genome-wide expression analysis identified IRAK1 expression as one of the two most powerful transcriptomic predictors of follicular lymphoma transformation to more aggressive disease [100]. Nonsynonymous IRAK1 polymorphisms that confer gain-of-function were identified in all cases of a cohort of Kaposi sarcoma-associated herpesvirus-positive primary effusion lymphoma patients where IRAK1 expression was required for tumor growth in culture [101]. TLR stimulation promotes proliferation in splenic marginal zone lymphoma samples in an IRAK1/4-dependent fashion [102]. TLR4 ligation similarly augments proliferation and therapy evasion in a multiple myeloma model [103]. A phase 2 trial reported that the IL-1 receptor antagonist Anakinra reduced proliferation and delayed progression to active disease by reducing downstream IL-6 induction in a subset of patients with smoldering or indolent myeloma [104].

ROLE OF INTERLEUKIN 1 RECEPTOR-ASSOCIATED KINASES IN SOLID TISSUE TUMORS

By and large, IRAK signaling has been studied in the context of normal and malignant leukocyte biology. However, an increasing body of evidence implicates IRAK kinase signaling as a critical effector of tumorigenesis in solid tissue cancers. IRAK4 signaling is a determinant of chemoresistance and disease progression in pancreatic and colorectal cancer [105,106], and IRAK4 expression is proposed to be a biomarker and predictor of poor prognosis in IDH wild-type and 1p19p nonco-deletion gliomas [107]. IRAK1 is purported to be a driver of oncogenesis or chemoresistance in head and neck cancers [108–110], breast cancer [111,112], hepatocellular carcinoma [113–115], nonsmall cell lung cancer [116,117], ovarian cancer [118], cervical cancer [119], melanoma [120], and gastric cancer [121]. IRAK1 is also implicated in supporting tumor-intrinsic resistance to radiation therapy [122] and IRAK1/4 inhibition has demonstrated adjunctive therapeutic benefit in preclinical models of anaplastic thyroid cancer and melanoma [123,124]. TLR9 overexpression is observed in prostate cancer, lending indirect support to the involvement in IRAK signaling in this malignancy as well [125]. A detailed dissection of IRAK signaling across solid tissue cancer is beyond the scope of this review; however, it is worth discussing several cases that highlight the oncogenic roles of IRAK2 and IRAK3. Evaluation of IRAK2 and IRAK3 is conspicuously absent in the hematologic malignancy arena, though the functions outlined below may be conserved features of malignant biology. IRAK3 is primarily recognized as a negative regulator of canonical Myddosome signaling and a mediator immunosuppressive cytokine production [41,47], and is being examined as an effector of tumorigenic immune evasion. Human lung

cancer lines induce IRAK3 expression in co-cultured monocytes, and IRAK3 deletion significantly reduces implanted tumor growth in mice, consistent with a role for IRAK3 in mollifying the antitumor response of tumor-associated macrophages [126]. This finding bolsters the observation that leukemic cells in CML induce IRAK3 expression in $CD14^+$ monocytes [89]. It would be worthwhile to assess the existence of such a phenomenon in AML and other leukemias that are notoriously refractory to conventional immunotherapies. Tumor-intrinsic epigenetic repression of IRAK3 is common in hepatocellular carcinoma and it is associated with a less favorable prognosis [127]. These findings indicate that IRAK3 may serve as a tumor suppressor by regulating signaling through the other IRAK family members.

IRAK2 is thought to be redundant with IRAK1 in mice, though the role of IRAK2 in human biology is less certain. It is nonetheless surprising that many reports advocate a tumor suppressor functionality for IRAK2. An IRAK2 SNP that promotes IRAK2 expression is associated with increased overall survival in NSCLC [128], whereas a nonsynonymous SNP that produces a hypofunctional IRAK2 isoform is associated with increased risk of death by colorectal cancer [129]. One study proposes that IRAK2 mitigates oncogenesis in colon cancer by phosphorylating the E3 ligase Smurf1 to trigger self-degradation [130]. Yu *et al.* [131] discovered that IRAK2 expression sensitizes oral squamous cell carcinoma to radiotherapy by instigating FADD (Fas-associated protein with death domain), an adaptor molecule involved in apoptotic signaling-driven apoptosis.

Notably, FADD contains a death domain (a uniting feature of Myddosome constituents), inviting the possibility that IRAK2 and other IRAK kinases participate in regulatory interactions with FADD. These models conflict with the notion that IRAK2 is a pseudokinase with activity that is confined to its constituency in the Myddosome and warrant further investigation of IRAK2 in the heme malignancies.

THERAPEUTIC APPROACHES TARGETING INTERLEUKIN 1 RECEPTOR-ASSOCIATED KINASES IN HUMAN CANCERS

Several parallel efforts to develop IRAK-targeted therapies for the hematological malignancies are ongoing (Table 1). Yet, IRAK inhibition as a treatment modality for these diseases is still a nascent concept. Several critical questions will need to be addressed before IRAK-targeted therapies achieve widespread clinical use. In this section, we address significant knowledge gaps, explicate the potential use of IRAK-targeted therapies in clinical practice, and highlight preliminary clinical trials.

Interleukin 1 receptor-associated kinase inhibitors as monotherapies

One of the major questions concerning IRAK-targeted therapies is whether they will demonstrate efficacy as monotherapies, and, if not, what an informed combination strategy would entail. Phase 1 trials with the compound CA-4948, a small molecule inhibitor with affinity for IRAK4 and FLT3 (Curis Inc), provide the best available data on this question. A phase 1 monotherapy trial in refractory non-Hodgkin's lymphoma (NCT03328078) reported a favorable safety profile and pharmacokinetic properties, with 8 of 28 patients experiencing a reduction in tumor burden of at least 20% [132]. An interim report from a phase 1 open-label trial (NCT04278768) using the same compound as a monotherapy in adults with AML or high-risk MDS described a reduction in bone marrow blasts in 10 of 12 patients who presented with elevated blast counts. Intriguingly, three of the four patients who achieved a complete response had mutations in the U2AF1 or SF3B1-splicing factors, which are known to drive disease by inducing hypermorphic IRAK4 isoforms [65,66]. These encouraging preliminary results bode well for the potential use of IRAK-targeted monotherapy in patients with splicing factor mutations and suggest efficacy in other genetic backgrounds. However, complementation with other drugs may be required for optimum effect in nonsplicing factor mutant AML/MDS. Kymera, operating under the logic that both IRAK4 loss-of-function and immunomodulatory imide drugs (IMiD) suppress NF-κB, have developed first-of-class 'IRAKIMiD' PROTACs. IRAKIMiDs consist of IRAK4 ligands coupled to an IMiD (lenalidomide or pomalidomide), thereby recruiting the E3 ligase cereblon to IRAK4 and the IMiD substrate transcription factors Ikaros and Aiolos to catalyze their ubiquitylation and proteasomal degradation. A candidate IRAKIMiD has demonstrated promise in preclinical MYD88-mutant lymphoma models with in-human trials planned for 2021 [133]. The absence of IRAK1-selective drugs in clinical trials makes it difficult to extrapolate the suitability of IRAK1 as a target for monotherapy or compare the relative utility of targeting IRAK1 over IRAK4. Pacritinib, a multikinase inhibitor of JAK2, FLT3, and IRAK1 developed as a treatment for myelofibrosis, demonstrated efficacy in a pilot phase 1 trial of FLT3-ITD AML [134]. Given the promiscuity of this compound, it is impossible to disentangle the individual contributions of IRAK1 and FLT3 inhibition to the observed

Table 1. Preclinical and clinical interleukin 1 receptor-associated kinase inhibitors

Compound	Source	Target	Mechanism	Disease Applications	Status
'23'	AstraZeneca	IRAK3 ($DC_{50} = 2$ nmol/l) IRAK4 ($IC_{50} = 5$ nmol/l)	PROTAC	Undetermined	Preclinical
'AZ1495'	AstraZeneca	IRAK1 ($IC_{50} = 24$ nmol/l)	Kinase inhibitor	Non-Hodgkin's lymphoma	Preclinical
BAY1830839	Bayer	IRAK4 ($IC_{50} = 3$ nmol/l)	Kinase inhibitor	Rheumatoid arthritis	Clinical
'Compound 9'	GlaxoSmithKline	IRAK4 ($DC_{50} = 36$ nmol/l)	PROTAC	Undetermined Non-Hodgkin's lymphoma	Preclinical
Emavusertib (CA-4948)	Curis	IRAK4 ($IC_{50} = 50$ nmol/l) FLT3	Kinase inhibitor	Waldenstrom's macroglobulenemia Acute myelogenous Leukemia Myelodysplastic syndrome Chronic lymphocytic Leukemia	Clinical
HS-243	Duke University	IRAK1 ($IC_{50} = 24$ nmol/l) IRAK4 ($IC_{50} = 200$ nmol/l)	Kinase inhibitor	Undetermined	Preclinical
IRAK-1–4 Inhibitor I	Amgen	IRAK1 ($IC_{50} = 300$ nmol/l)	Kinase inhibitor	Not applicable	Preclinical
JH-X-119-01	Dana Farber Cancer Institute	IRAK1 ($IC_{50} = 9$ nmol/l)	Kinase inhibitor	Non-Hodgkin's lymphoma	Preclinical
JNJ-1013	Janssen Pharmaceuticals	IRAK1 ($DC_{50} = 3$ nmol/l)	PROTAC	Non-Hodgkin's lymphoma Hidradenitis suppurtiva	Preclinical
KT-474	Kymera Therapeutics	IRAK4 ($DC_{50} = 2.1$ nmol/l)	PROTAC	Atopic dermatitis Non-Hodgkin's lymphoma	Clinical
NCGC1481	Kurome Therapeutics	IRAK4 ($IC_{50} = 0.8$ nmol/l) IRAK1 ($IC_{50} = 22.6$ nmol/l) FLT3 ($IC_{50} < 0.5$ nmol/l)	Kinase inhibitor	Myelodysplastic syndrome Acute myelogenous leukemia	Preclinical
Pacritinib	CTI Biopharma	JAK2 ($IC_{50} = 23$ nmol/l) FLT3 ($IC_{50} = 22$ nmol/l) IRAK1 ($IC_{50} < 20$ nmol/l) IRAK4	Kinase inhibitor	Myeloproliferative neoplasms Myelodysplastic syndrome	Clinical
R289	Rigel Pharmaceuticals	IRAK1 IRAK4	Kinase inhibitor	Rheumatological diseases	Clinical
Zabedosertib (BAY1834845)	Bayer	IRAK4 ($IC_{50} = 3.4$ nmol/l)	Kinase inhibitor	Rheumatological diseases	Clinical
Zimlovisertib (PF-06650833)	Pfizer	IRAK4 ($IC_{50} = 0.2$ nmol/l)	Kinase inhibitor	Rheumatoid arthritis	Clinical

response. However, one preclinical study reported that pacritinib exhibited potency against primary AML samples of diverse genetic backgrounds, whereas the efficacy of a FLT3 inhibitor lacking affinity for IRAK1 was restricted to samples harboring FLT3-ITD [80]. This study concords with the results of a phase 3 trial in myelofibrosis that identified a response to pacritinib regardless of $JAK2^{V617F}$ allele burden [135]. Further, recently generated IRAK1 inhibitors and PROTACS are effective in constraining MYD88 mutant lymphomas *in vitro* [94,99▪]. These results establish a precedent for the clinical development of IRAK1 inhibitors and intimate that pacritinib, by virtue of its activity against

IRAK, may be a useful drug in the hematologic malignancies regardless of mutational background.

The matter of which drugs to pair with IRAK-targeted therapy captures two related issues; first, the resistance mechanisms to IRAK-targeted therapies are largely unknown, and; second that recruitment of IRAK signaling may underly adaptive resistance to other therapies. Parallel IRAK signaling and BCR-BTK signaling converge on NF-κB in B-cell lymphomas [92,136], justifying the combination of IRAK-targeted therapies with a BTK inhibitor. The synergy of IRAK inhibitors with the BTK inhibitor ibrutinib affirmed in multiple preclinical studies [93–95, 137,138], and NCT03328078 has expanded to enroll refractory lymphoma patients for dual therapy with CA-4948 and ibrutinib. Rhyasen *et al.* [64] identified upregulation of the antiapoptotic factor BCL2 in MDS/AML clones that escaped IRAK1 inhibitor treatment, with a synergistic reduction of cell expansion and viability obtained upon the addition of BCL2 inhibitors. Similarly, BCL2 inhibitor supplementation dramatically augmented a meager response to single-agent IRAK1/4 inhibitor treatment in a xenograft model of T-ALL [139]. This result invites a potential synergistic combination of IRAK-targeted therapies with the BCL2 inhibitor venetoclax. Indeed, the trial with CA-4948 in AML and high-risk AML (NCT04278768) now includes a venetoclax dual therapy arm. Preliminary results from this arm have not been released at the time of publication. BCL2 upregulation remains the only described adaptation to IRAK inhibitor treatment in AML/MDS; a thorough delineation of escape mechanisms is an eminent need for future studies.

Interleukin 1 receptor-associated kinase inhibitors to overcome adaptive resistance mechanisms to therapy

An expanding body of evidence implicates IRAK signaling as a mechanism of adaptive resistance to various forms of cancer therapy, indicating that IRAK-targeted drugs may realize widespread application as adjunctive therapies. IRAK signaling promotes chemoresistance in diverse preclinical solid-tissue cancer models in which IRAK inhibition restores sensitivity to SN-38, 5-FU, oxaliplatin, gemcitabine, paclitaxel, sorafenib, doxorubicin, and the irinotecan metabolite SN-P38 [105,106,109,112, 113,140,141]. Recent investigations assert IRAK signaling as a mechanism of adaptive resistance in the hematologic malignancies as well. Melgar *et al.* [142] performed kinome screening and gene expression analysis in FLT3-ITD mutant AML cell lines treated with clinical FLT3 inhibitors and revealed activation of IRAK-mediated innate immune signaling to be a mechanism of acute adaptation to FLT3 inhibition. A novel IRAK1/IRAK4/FLT3 tri-selective inhibitor eradicated adaptively resistant AML clones in liquid culture and xenografts [142,143]. Two subsequent studies reiterate innate immune activation [144] and upregulation of the TLR ligand S100A9 [145], respectively, as mediators of adaptive response to the FLT3 inhibition in AML. Du *et al.* [146▪] report that treatment with Ara-C or anthracyclines induces a subpopulation of leukemic cells to acquire an inflammatory signature and senescence-like state that enables chemoresistance and disease recurrence. Although the IRAK kinases were not explicitly evaluated, the inflammatory signatures in the resistant leukemic population are enriched for pathways regulated by the IRAK kinases.

No inhibitors or degraders of IRAK2 or IRAK3 are currently being vetted for therapeutic application, though an IRAK3-selective PROTAC has recently been developed [147]. It will be fascinating to see whether targeting IRAK3 demonstrates broad utility in stimulating antitumor immune responses, possibly as part of a combination with checkpoint inhibitors as proposed elsewhere [148].

CONCLUSION

An extensive body of literature implicating IRAK signaling in the hematologic malignancies and promising preliminary clinical trial data validate IRAK-targeted therapy as an exciting approach to the hematologic malignancies. However, these therapies were constructed on an incomplete model of IRAK signaling in cancer. The data reviewed above conflict with the convention that IRAK4 and IRAK1 signal in a linear and interdependent fashion. Moreover, the current conception of IRAK2-signaling functions is problematically gleaned from mouse models, and IRAK2 functionality in the human system is nebulous. These conspicuous gaps in the literature cast doubt as to whether extant IRAK inhibitors are fully optimized for clinical use. Future investigations must address the contribution of IRAK1/IRAK4 scaffolding functions, refine the role of IRAK2 in human malignancy, and evaluate the capacity for signaling redundancy among IRAK family members. These studies are direly needed to settle debate concerning the prioritization of IRAK degraders or ATP-competitive inhibitors, inform ideal target selection, and resolve the demand for a multikinase approach, respectively.

Acknowledgements

We would like to thank members of the Starczynowski labs for helpful suggestions and editing of the review. Figures and illustrations were inspired by BioRender.com.

Financial support and sponsorship

D.T.S. is supported by Cincinnati Children's Hospital Research Foundation, Leukemia Lymphoma Society, National Institute of Health (R35HL135787, R01DK102759, R01DK113639), The Edward P Evans Foundation, and Cancer Free Kids grants. J.B. is supported by National Institutes of Health T32CA117846-04.

Conflicts of interest

D.T.S. serves on the scientific advisory board at Kurome Therapeutics, and is a consultant for Kymera Therapeutics, Kurome Therapeutics, Captor Therapeutics, and Tolero Therapeutics. D.T.S. has equity in Kurome Therapeutics. The other authors declare no competing financial interests.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Paracatu LC, Schuettpelz LG. Contribution of aberrant toll like receptor signaling to the pathogenesis of myelodysplastic syndromes. Front Immunol 2020; 11:1236.
2. Sallman DA, List A. The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. Blood 2019; 133:1039–1048.
3. Barreyro L, Chlon TM, Starczynowski DT. Chronic immune response dysregulation in MDS pathogenesis. Blood 2018; 132:1553–1560.
4. Gañán-Gómez I, Wei Y, Starczynowski DT, *et al.* Deregulation of innate immune and inflammatory signaling in myelodysplastic syndromes. Leukemia 2015; 29:1458–1469.
5. Trowbridge JJ, Starczynowski DT. Innate immune pathways and inflammation in hematopoietic aging, clonal hematopoiesis, and MDS. J Exp Med 2021; 218:e20201544.
6. Varney ME, Melgar K, Niederkorn M, *et al.* Deconstructing innate immune signaling in myelodysplastic syndromes. Exp Hematol 2015; 43:587–598.
7. Monlish DA, Bhatt ST, Schuettpelz LG. The role of Toll-like receptors in hematopoietic malignancies. Front Immunol 2016; 7:390.
8. Ratajczak MZ, Bujko K, Cymer M, *et al.* The Nlrp3 inflammasome as a 'rising star' in studies of normal and malignant hematopoiesis. Leukemia 2020; 34:1512–1523.
9. Ivy KS, Brent Ferrell P. Disordered immune regulation and its therapeutic targeting in myelodysplastic syndromes. Curr Hematol Malig Rep 2018; 13:244–255.
10. Li S, Strelow A, Fontana EJ, Wesche H. IRAK-4: a novel member of the IRAK family with the properties of an IRAK-kinase. Proc Natl Acad Sci U S A 2002; 99:5567–5572.
11. O'Neill LAJ, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol 2007; 7:353–364.
12. Gay NJ, Gangloff M, Weber ANR. Toll-like receptors as molecular switches. Nat Rev Immunol 2006; 6:693–698.
13. Wesche H, Henzel WJ, Shillinglaw W, *et al.* MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. Immunity 1997; 7:837–847.
14. Lin SC, Lo YC, Wu H. Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling. Nature 2010; 465:885–890.
15. Moncrieffe MC, Bollschweiler D, Li B, *et al.* MyD88 death-domain oligomerization determines myddosome architecture: implications for Toll-like receptor signaling. Structure 2020; 28:281.e3–289.e3.
16. Ferrao R, Zhou H, Shan Y, *et al.* IRAK4 dimerization and trans-autophosphorylation are induced by Myddosome assembly. Mol Cell 2014; 55:891–903.
17. Kollewe C, Mackensen AC, Neumann D, *et al.* Sequential autophosphorylation steps in the interleukin-1 receptor-associated kinase-1 regulate its availability as an adapter in interleukin-1 signaling. J Biol Chem 2004; 279:5227–5236.
18. Vollmer S, Strickson S, Zhang T, *et al.* The mechanism of activation of IRAK1 and IRAK4 by interleukin-1 and Toll-like receptor agonists. Biochem J 2017; 474:2027–2038.
19. Qin J, Jiang Z, Qian Y, *et al.* IRAK4 kinase activity is redundant for interleukin-1 (IL-1) receptor-associated kinase phosphorylation and IL-1 responsiveness. J Biol Chem 2004; 279:26748–26753.
20. Song KW, Talamas FX, Suttmann RT, *et al.* The kinase activities of interleukin-1 receptor associated kinase (IRAK)-1 and 4 are redundant in the control of inflammatory cytokine expression in human cells. Mol Immunol 2009; 46:1458–1466.
21. Wang L, Qiao Q, Ferrao R, *et al.* Crystal structure of human IRAK1. Proc Natl Acad Sci U S A 2017; 114:13507–13512.
22. Zhang J, Fu L, Shen B, *et al.* Assessing IRAK4 functions in ABC DLBCL by IRAK4 kinase inhibition and protein degradation. Cell Chem Biol 2020; 27:1500.e13–1509.e13.
23. Cushing L, Winkler A, Jelinsky SA, *et al.* IRAK4 kinase activity controls Toll-like receptor–induced inflammation through the transcription factor IRF5 in primary human monocytes. J Biol Chem 2017; 292:18689–18698.
24. Hjorton K, Hagberg N, Israelsson E, *et al.* Cytokine production by activated plasmacytoid dendritic cells and natural killer cells is suppressed by an IRAK4 inhibitor. Arthritis Res Ther 2018; 20:1–11.
25. Sun J, Li N, Oh KS, *et al.* Comprehensive RNAi-based screening of human and mouse TLR pathways identifies species-specific preferences in signaling protein use. Sci Signal 2016; 9:ra3.
26. Emmerich CH, Ordureau A, Strickson S, *et al.* Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. Proc Natl Acad Sci U S A 2013; 110:15247–15252.
27. Martin MU, Wesche H. Summary and comparison of the signaling mechanisms of the Toll/interleukin-1 receptor family. Biochim Biophys Acta 2002; 1592:265–280.
28. Conze DB, Wu C-J, Thomas JA, *et al.* Lys63-linked polyubiquitination of IRAK-1 is required for interleukin-1 receptor- and toll-like receptor-mediated NF-κB activation. Mol Cell Biol 2008; 28:3538–3547.
29. Lin KM, Hu W, Troutman TD, *et al.* IRAK-1 bypasses priming and directly links TLRs torapid NLRP3 inflammasome activation. Proc Natl Acad Sci U S A 2014; 111:775–780.
30. Fernandes-Alnemri T, Kang S, Anderson C, *et al.* Cutting edge: TLR signaling licenses IRAK1 for rapid activation of the NLRP3 inflammasome. J Immunol 2013; 191:3995–3999.
31. Humphries F, Bergin R, Jackson R, *et al.* The E3 ubiquitin ligase Pellino2 mediates priming of the NLRP3 inflammasome. Nat Commun 2018; 9:1560.
32. Janssens S, Beyaert R. Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members. Mol Cell 2003; 11:293–302.
33. Wesche H, Gao X, Li X, *et al.* IRAK-M is a novel member of the pelle/interleukin-1 receptor-associated kinase (IRAK) family. J Biol Chem 1999; 274:19403–19410.
34. Ye H, Arron JR, Lamothe B, *et al.* Distinct molecular mechanism for initiating TRAF6 signalling. Nature 2002; 418:443–447.
35. Muzio M, Ni J, Feng P, *et al.* (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL- 1 signaling. Science 1997; 278:1612–1615.
36. Keating SE, Maloney GM, Moran EM, Bowie AG. IRAK-2 participates in multiple Toll-like receptor signaling pathways to NFκB via activation of TRAF6 ubiquitination. J Biol Chem 2007; 282:33435–33443.
37. Kawagoe T, Sato S, Matsushita K, *et al.* Sequential control of Toll-like receptor-dependent responses by IRAK1 and IRAK2. Nat Immunol 2008; 9:684–691.
38. Pauls E, Nanda SK, Smith H, *et al.* Two phases of inflammatory mediator production defined by the study of IRAK2 and IRAK1 knock-in mice. J Immunol 2013; 191:2717–2730.
39. Wan Y, Xiao H, Affolter J, *et al.* Interleukin-1 receptor-associated kinase 2 is critical for lipopolysaccharide-mediated posttranscriptional control. J Biol Chem 2009; 284:10367–10375.
40. Zhou H, Bulek K, Li X, *et al.* IRAK2 directs stimulus-dependent nuclear export of inflammatory mRNAs. Elife 2017; 6:e29630.
41. Kobayashi K, Hernandez LD, Galán JE, *et al.* IRAK-M is a negative regulator of Toll-like receptor signaling. Cell 2002; 110:191–202.
42. Du J, Nicolaes GAF, Kruijswijk D, *et al.* The structure function of the death domain of human IRAK-M. Cell Commun Signal 2014; 12:1–19.
43. Freihat LA, Wheeler JI, Wong A, Turek I, *et al.* IRAK3 modulates downstream innate immune signalling through its guanylate cyclase activity. Sci Rep 2019; 9:1–12.
44. van 't Veer C, van den Pangaart PS, van Zoelen MAD, *et al.* Induction of IRAK-M Is associated with lipopolysaccharide tolerance in a human endotoxemia model. J Immunol 2007; 179:7110–7120.
45. Balaci L, Spada MC, Olla N, *et al.* IRAK-M is involved in the pathogenesis of early-onset persistent asthma. Am J Hum Genet 2007; 80:1103–1114.
46. Lange SM, Nelen MI, Cohen P, Kulathu Y. Dimeric structure of the pseudokinase IRAK3 suggests an allosteric mechanism for negative regulation. Structure 2021; 29:238.e4–251.e4.
47. Zhou H, Yu M, Fukuda K, *et al.* IRAK-M mediates Toll-like receptor/IL-1R-induced NFκB activation and cytokine production. EMBO J 2013; 32:583–596.
48. Cazzola M. Myelodysplastic syndromes. N Engl J Med 2020; 383: 1358–1374.
49. Abdel-Wahab O, Gao J, Adli M, *et al.* Deletion of Asxl1 results in myelodysplasia and severe developmental defects in vivo. J Exp Med 2013; 210:2641–2659.

50. Schneider RK, Schenone M, Ferreira MV, *et al.* Rps14 haploinsufficiency causes a block in erythroid differentiation mediated by S100A8 and S100A9. Nat Med 2016; 22:288–297.
51. Starczynowski DT, Kuchenbauer F, Argiropoulos B, *et al.* Identification of miR-145 and miR-146a as mediators of the 5q-syndrome phenotype. Nat Med 2010; 16:49–58.
52. Varney ME, Niederkorn M, Konno H, *et al.* Loss of Tifab, a del(5q) MDS gene, alters hematopoiesis through derepression of Toll-like receptor-TRAF6 signaling. J Exp Med 2015; 212:1967–1985.
53. Cull AH, Snetsinger B, Buckstein R, *et al.* Tet2 restrains inflammatory gene expression in macrophages. Exp Hematol 2017; 55:56.e13–70.e13.
54. Hamarsheh S, Osswald L, Saller BS, *et al.* Oncogenic KrasG12D causes myeloproliferation via NLRP3 inflammasome activation. Nat Commun 2020; 11:1–17.
55. Zhao JL, Rao DS, Boldin MP, *et al.* NF-κB dysregulation in microRNA-146a-deficient mice drives the development of myeloid malignancies. Proc Natl Acad Sci U S A 2011; 108:9184–9189.
56. Fang J, Barker B, Bolanos L, *et al.* Myeloid malignancies with chromosome 5q deletions acquire a dependency on an intrachromosomal NF-κB gene network. Cell Rep 2014; 8:1328–1338.
57. Varney ME, Choi K, Bolanos L, *et al.* Epistasis between TIFAB and miR-146a: neighboring genes in del(5q) myelodysplastic syndrome. Leukemia 2017; 31:491–495.
58. Fang J, Bolanos LC, Choi K, *et al.* Ubiquitination of hnRNPA1 by TRAF6 links chronic innate immune signaling with myelodysplasia. Nat Immunol 2017; 18:236–245.
59. Wei Y, Dimicoli S, Bueso-Ramos C, *et al.* Toll-like receptor alterations in myelodysplastic syndrome. Leukemia 2013; 27:1832–1840.
60. Dimicoli S, Wei Y, Bueso-Ramos C, *et al.* Overexpression of the Toll-Like receptor (TLR) signaling adaptor MYD88, but lack of genetic mutation, in myelodysplastic syndromes. PLoS One 2013; 8:e71120.
61. Maratheftis CI, Andreakos E, Moutsopoulos HM, Voulgarelis M. Toll-like receptor-4 is up-regulated in hematopoietic progenitor cells and contributes to increased apoptosis in myelodysplastic syndromes. Clin Cancer Res 2007; 13:1154–1160.
62. Pellagatti A, Cazzola M, Giagounidis A, *et al.* Deregulated gene expression pathways in myelodysplastic syndrome hematopoietic stem cells. Leukemia 2010; 24:756–764.
63. Hofmann WK, De Vos S, Komor M, *et al.* Characterization of gene expression of CD34 + cells from normal and myelodysplastic bone marrow. Blood 2002; 100:3553–3560.
64. Rhyasen GW, Bolanos L, Fang J, *et al.* Targeting IRAK1 as a therapeutic approach for myelodysplastic syndrome. Cancer Cell 2013; 24:90–104.
65. Smith MA, Choudhary GS, Pellagatti A, *et al.* U2AF1 mutations induce oncogenic IRAK4 isoforms and activate innate immune pathways in myeloid malignancies. Nat Cell Biol 2019; 21:640–650.
66. Choudhary GS, Smith MA, Pellagatti A, *et al.* SF3B1 mutations induce oncogenic IRAK4 isoforms and activate targetable innate immune pathways in MDS and AML. Blood 2019; 134:4224.
67. Zambetti NA, Ping Z, Chen S, *et al.* Mesenchymal inflammation drives genotoxic stress in hematopoietic stem cells and predicts disease evolution in human preleukemia. Cell Stem Cell 2016; 19:613–627.
68. Chen X, Eksioglu EA, Zhou J, *et al.* Induction of myelodysplasia by myeloid-derived suppressor cells. J Clin Invest 2013; 123:4595–4611.
69. Cheng P, Corzo CA, Luetteke N, *et al.* Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. J Exp Med 2008; 205:2235–2249.
70. Basiorka AA, McGraw KL, Eksioglu EA, *et al.* The NLRP3 inflammasome functions as a driver of the myelodysplastic syndrome phenotype. Blood 2016; 128:2960–2975.
71. Sallman DA, Cluzeau T, Basiorka AA, List A. Unraveling the pathogenesis of MDS: The NLRP3 inflammasome and pyroptosis drive the MDS phenotype. Front Oncol 2016; 6:151.
72. ■ Chakraborty S, Shapiro LC, de Oliveira S, *et al.* Therapeutic targeting of the inflammasome in myeloid malignancies. Blood Cancer J 2021; 11:1–10.
S100A8/9 alarmins induce hematopoietic dysfunction in MDS by triggering NLRP3 inflammasome.
73. Kittang AO, Kordasti S, Sand KE, *et al.* Expansion of myeloid derived suppressor cells correlates with number of T regulatory cells and disease progression in myelodysplastic syndrome. Oncoimmunology 2016; 5:e1062208.
74. Sinha P, Okoro C, Foell D, *et al.* Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells. J Immunol 2008; 181:4666–4675.
75. He Y, Hara H, Núñez G. Mechanism and regulation of NLRP3 inflammasome activation. Trends Biochem Sci 2016; 41:1012–1021.
76. Carey A, Edwards DK, Eide CA, *et al.* Identification of interleukin-1 by functional screening as a key mediator of cellular expansion and disease progression in acute myeloid leukemia. Cell Rep 2017; 18:3204–3218.
77. Muto T, Walker CS, Choi K, *et al.* Adaptive response to inflammation contributes to sustained myelopoiesis and confers a competitive advantage in myelodysplastic syndrome HSCs. Nat Immunol 2020; 21:535–545.
78. ■ Wang T, Pine AR, Kotini AG, *et al.* Sequential CRISPR gene editing in human iPSCs charts the clonal evolution of myeloid leukemia and identifies early disease targets. Cell Stem Cell 2021; 28:1074.e7–1089.e7.
IRAK1/IRAK4 dual inhibitors suppressed progenitor function in leukemic, but not parental, iPSC-derived hematopoietic cells.
79. Li J, Volk A, Zhang J, *et al.* Sensitizing leukemia stem cells to NF-κB inhibitor treatment in vivo by inactivation of both TNF and IL-1 signaling. Oncotarget 2017; 8:8420.
80. Hosseini MM, Kurtz SE, Abdelhamed S, *et al.* Inhibition of interleukin-1 receptor-associated kinase-1 is a therapeutic strategy for acute myeloid leukemia subtypes. Leukemia 2018; 32:2374–2387.
81. Martínez-Trillos A, Pinyol M, Navarro A, *et al.* Mutations in TLR/MYD88 pathway identify a subset of young chronic lymphocytic leukemia patients with favorable outcome. Blood 2014; 123:3790–3796.
82. Puente XS, Pinyol M, Quesada V, *et al.* Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature 2011; 475:101–105.
83. Landau DA, Carter SL, Stojanov P, *et al.* Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. Cell 2013; 152:714–726.
84. Giménez N, Schulz R, Higashi M, *et al.* Targeting IRAK4 disrupts inflammatory pathways and delays tumor development in chronic lymphocytic leukemia. Leukemia 2020; 34:100–114.
85. Improgo MR, Tesar B, Klitgaard JL, *et al.* MYD88 L265P mutations identify a prognostic gene expression signature and a pathway for targeted inhibition in CLL. Br J Haematol 2019; 184:925–936.
86. Dussiau C, Trinquand A, Lhermitte L, *et al.* Targeting IRAK1 in T-Cell acute lymphoblastic leukemia. Oncotarget 2015; 6:18956.
87. Pei JS, Chang WS, Hsu PC, *et al.* Significant association βetween the mIR146a genotypes and susceptibility to childhood acute lymphoblastic leukemia in Taiwan. Cancer Genomics Proteomics 2020; 17:175–180.
88. Dadashian EL, McAuley EM, Liu D, *et al.* TLR signaling is activated in lymph node–resident CLL cells and is only partially inhibited by ibrutinib. Cancer Res 2019; 79:360–371.
89. del Fresno C, Otero K, Gómez-García L, *et al.* Tumor cells deactivate human monocytes by up-regulating IL-1 receptor associated kinase-M expression via CD44 and TLR4. J Immunol 2005; 174:3032–3040.
90. Poulain S, Roumier C, Decambron A, *et al.* MYD88 L265P mutation in Waldenstrom macroglobulinemia. Blood 2013; 121:4504–4511.
91. Treon SP, Xu L, Yang G, *et al.* MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. N Engl J Med 2012; 367:826–833.
92. Ngo VN, Young RM, Schmitz R, *et al.* Oncogenically active MYD88 mutations in human lymphoma. Nature 2011; 470:115–119.
93. Yang G, Liu X, Chen J, *et al.* Targeting IRAK1/IRAK4 signaling in Waldenstrom's macroglobulinemia. Blood 2015; 126:404–1404.
94. Hatcher JM, Yang G, Wang L, *et al.* Discovery of a selective, covalent IRAK1 inhibitor with antiproliferative activity in MYD88 mutated B-cell lymphoma. ACS Med Chem Lett 2020; 11:2238–2243.
95. Scott JS, Degorce SL, Anjum R, *et al.* Discovery and optimization of pyrrolopyrimidine inhibitors of interleukin-1 receptor associated kinase 4 (IRAK4) for the treatment of mutant MYD88 L265P diffuse large B-cell lymphoma. J Med Chem 2017; 60:10071–10091.
96. Yang G, Hatcher J, Wang J, *et al.* A novel, highly selective IRAK1 inhibitor Jh-X-119-01 shows synergistic tumor cell killing with ibrutinib in MYD88 mutated B-cell lymphoma cells. Blood 2017; 130:719.
97. Phelan JD, Young RM, Webster DE, *et al.* A multiprotein supercomplex controlling oncogenic signalling in lymphoma. Nature 2018; 560:387–391.
98. Reddy A, Zhang J, Davis NS, *et al.* Genetic and functional drivers of diffuse large B cell lymphoma. Cell 2017; 171:481–494.
99. ■ Fu L, Zhang J, Shen B, *et al.* Discovery of highly potent and selective IRAK1 degraders to probe scaffolding functions of IRAK1 in ABC DLBCL. J Med Chem 2021; 64:10878–10889.
IRAK1 PROTACS exhibited antiproliferative potency against ABC DLBCL.
100. Brodtkorb M, Lingjærde OC, Huse K, *et al.* Whole-genome integrative analysis reveals expression signatures predicting transformation in follicular lymphoma. Blood 2014; 123:1051–1054.
101. Yang D, Chen W, Xiong J, *et al.* Interleukin 1 receptor-associated kinase 1 (IRAK1) mutation is a common, essential driver for Kaposi sarcoma herpesvirus lymphoma. Proc Natl Acad Sci USA 2014; 111:E4762–E4768.
102. Fonte E, Agathangelidis A, Reverberi D, *et al.* Toll-like receptor stimulation in splenic marginal zone lymphoma can modulate cell signaling, activation and proliferation. Haematologica 2015; 100:1460–1468.
103. Bao H, Lu P, Li Y, *et al.* Triggering of toll-like receptor-4 in human multiple myeloma cells promotes proliferation and alters cell responses to immune and chemotherapy drug attack. Cancer Biol Ther 2011; 11:58–67.
104. Lust JA, Lacy MQ, Zeldenrust SR, *et al.* Induction of a chronic disease state in patients with smoldering or indolent multiple myeloma by targeting interleukin 1β-induced interleukin 6 production and the myeloma proliferative component. Mayo Clin Proc 2009; 84:114–122.
105. Zhang D, Li L, Jiang H, *et al.* Tumor–Stroma IL1b-IRAK4 feedforward circuitry drives tumor fibrosis, chemoresistance, and poor prognosis in pancreatic cancer. Cancer Res 2018; 78:1700–1712.
106. Li Q, Chen Y, Zhang D, *et al.* IRAK4 mediates colitis-induced tumorigenesis and chemoresistance in colorectal cancer. JCI Insight 2019; 4:e130867.

107. Wang J, Liu B, Yao J, *et al.* Interleukin-1 receptor-associated kinase 4 as a potential biomarker: overexpression predicts poor prognosis in patients with glioma. Oncol Lett 2021; 21:1–11.

108. Meng DF, Sun R, Liu GY, *et al.* S100A14 suppresses metastasis of nasopharyngeal carcinoma by inhibition of NF-kB signaling through degradation of IRAK1. Oncogene 2020; 39:5307–5322.

109. Liu L, Liu S, Deng P, *et al.* Targeting the IRAK1-S100A9 axis overcomes resistance to paclitaxel in nasopharyngeal Carcinoma. Cancer Res 2021; 81:1413–1425.

110. Adams AK, Bolanos LC, Dexheimer PJ, *et al.* IRAK1 is a novel DEK transcriptional target and is essential for head and neck cancer cell survival. Oncotarget 2015; 6:43395.

111. Goh JY, Feng M, Wang W, *et al.* Chromosome 1q21.3 amplification is a trackable biomarker and actionable target for breast cancer recurrence. Nat Med 2017; 23:1319–1330.

112. Wee ZN, Yatim SMJM, Kohlbauer VK, *et al.* IRAK1 is a therapeutic target that drives breast cancer metastasis and resistance to paclitaxel. Nat Commun 2015; 6:8746.

113. Cheng BY, Lau EY, Leung HW, *et al.* Irak1 augments cancer stemness and drug resistance via the ap-1/akr1b10 signaling cascade in hepatocellular carcinoma. Cancer Res 2018; 78:2332–2342.

114. Kim SY, Shen Q, Son K, *et al.* SMARCA4 oncogenic potential via IRAK1 enhancer to activate Gankyrin and AKR1B10 in liver cancer. Oncogene 2021; 40:4652–4662.

115. Ye ZH, Gao L, Wen DY, *et al.* Diagnostic and prognostic roles of IRAK1 in hepatocellular carcinoma tissues: an analysis of immunohistochemistry and RNA-sequencing data from the cancer genome atlas. Onco Targets Ther 2017; 10:1711.

116. Behrens C, Feng L, Kadara H, *et al.* Expression of interleukin-1 receptor-associated kinase-1 in nonsmall cell lung carcinoma and preneoplastic lesions. Clin Cancer Res 2010; 16:34–44.

117. Liu YN, Tsai MF, Wu SG, *et al.* miR-146b-5p enhances the sensitivity of NSCLC to EGFR tyrosine kinase inhibitors by regulating the IRAK1/NF-κB pathway. Mol Ther Nucleic Acids 2020; 22:471–483.

118. Raval N, Mohammadzadeh D, Nguyen T. Targeting interleukin-1 receptor-associated kinase 1 (IRAK1) in ovarian cancer. FASEB J 2021; 35.

119. Hu Q, Song J, Ding B, *et al.* miR-146a promotes cervical cancer cell viability via targeting IRAK1 and TRAF6. Oncol Rep 2018; 39:3015–3024.

120. Boukerche H, Su ZZ, Kang DC, Fisher PB. Identification and cloning of genes displaying elevated expression as a consequence of metastatic progression in human melanoma cells by rapid subtraction hybridization. Gene 2004; 343:191–201.

121. Ranjbar R, Hesari AR, Ghasemi F, Sahebkar A. Expression of microRNAs and IRAK1 pathway genes are altered in gastric cancer patients with Helicobacter pylori infection. J Cell Biochem 2018; 119:7570–7576.

122. Liu PH, Shah RB, Li Y, *et al.* An IRAK1–PIN1 signalling axis drives intrinsic tumour resistance to radiation therapy. Nat Cell Biol 2019; 21:203–213.

123. Kawamura Y, Saijo K, Imai H, Ishioka C. Inhibition of IRAK1/4 enhances the antitumor effect of lenvatinib in anaplastic thyroid cancer cells. Cancer Sci 2021. [Epub ahead of print]

124. Srivastava R, Geng D, Liu Y, *et al.* Augmentation of therapeutic responses in melanoma by inhibition of IRAK-1,-4. Cancer Res 2012; 72:6209–6216.

125. Väisänen MR, Väisänen T, Jukkola-Vuorinen A, *et al.* Expression of toll-like receptor-9 is increased in poorly differentiated prostate tumors. Prostate 2010; 70:817–824.

126. Standiford TJ, Kuick R, Bhan U, *et al.* TGF-B-induced IRAK-M expression in tumor-associated macrophages regulates lung tumor growth. Oncogene 2011; 30:2475–2484.

127. Kuo CC, Shih YL, Su HY, *et al.* Methylation of IRAK3 is a novel prognostic marker in hepatocellular carcinoma. World J Gastroenterol 2015; 21:3960.

128. Xu Y, Liu H, Liu S, *et al.* Genetic variant of IRAK2 in the toll-like receptor signaling pathway and survival of nonsmall cell lung cancer. Int J Cancer 2018; 143:400–2408.

129. Wang H, Flannery SM, Dickhöfer S, *et al.* A coding IRAK2 protein variant compromises toll-like receptor (TLR) signaling and is associated with colorectal cancer survival. J Biol Chem 2014; 289:23123–23131.

130. Liu J, Chen Y, Huang Q, *et al.* IRAK2 counterbalances oncogenic Smurf1 in colon cancer cells by dictating ER stress. Cell Signal 2018; 48:69–80.

131. Yu CC, Chan MWY, Lin HY, *et al.* IRAK2, an IL1R/TLR immune mediator, enhances radiosensitivity via modulating caspase 8/3-mediated apoptosis in oral squamous cell carcinoma. Front Oncol 2021; 11:2361.

132. Nowakowski GS, Leslie LA, Younes A, *et al.* Safety, pharmacokinetics and activity of CA-4948, an IRAK4 inhibitor, for treatment of patients with relapsed or refractory hematologic malignancies: results from the phase 1 study. Blood 2020; 136:44–45.

133. Lue JK, Manavalan JS, Klaus C, *et al.* Targeting MYD88-mutant DLBCL with IRAKIMiDs: a comparison to IRAK4 kinase inhibition and evaluation of synergy with rational combinations. Blood 2020; 136:12.

134. Jeon JY, Zhao Q, Buelow DR, *et al.* Preclinical activity and a pilot phase I study of pacritinib, an oral JAK2/FLT3 inhibitor, and chemotherapy in FLT3-ITD-positive AML. Invest New Drugs 2020; 38:340–349.

135. Verstovsek S, Scott BL, Taylor JA, Mascarenhas J. The oral JAK2/IRAK1 inhibitor Pacritinib demonstrates spleen volume reduction in myelofibrosis patients independent of JAK2V617F allele burden. Blood 2019; 134:1674-1674.

136. Davis RE, Ngo VN, Lenz G, *et al.* Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature 2010; 463:88–92.

137. Booher RN, Nowakowski GS, Patel K, *et al.* Preclinical activity of IRAK4 kinase inhibitor CA-4948 alone or in combination with targeted therapies and preliminary phase 1 clinical results in non-Hodgkin lymphoma. Blood 2018; 132:4168.

138. Choudhary D, Wood N, Romero DL, *et al.* Synergistic blockade of activated b cell-like DLBCL proliferation with a selective inhibitor of IRAK4 in combination with inhibition of the b-cell receptor signaling network. Blood 2013; 122:3833.

139. Li Z, Younger K, Gartenhaus R, *et al.* Inhibition of IRAK1/4 sensitizes T cell acute lymphoblastic leukemia to chemotherapies. J Clin Invest 2015; 125:1081–1097.

140. Zhang D, Li L, Jiang H, *et al.* Constitutive IRAK4 activation underlies poor prognosis and chemoresistance in pancreatic ductal adenocarcinoma. Clin Cancer Res 2017; 23:1748–1759.

141. Dodhiawala PB, Khurana N, Zhang D, *et al.* TPL2 enforces RAS-induced inflammatory signaling and is activated by point mutations. J Clin Invest 2020; 130:4771–4790.

142. Melgar K, Walker MM, Jones LQM, *et al.* Overcoming adaptive therapy resistance in AML by targeting immune response pathways. Sci Transl Med 2019; 11.

143. Jones LQM, Melgar K, Bolanos L, *et al.* Targeting AML-associated FLT3 mutations with a type I kinase inhibitor. J Clin Invest 2020; 130:2017–2023.

144. Gebru MT, Atkinson JM, Young MM, *et al.* Glucocorticoids enhance the antileukemic activity of FLT3 inhibitors in FLT3-mutant acute myeloid leukemia. Blood 2020; 136:1067–1079.

145. Thomas MEZ, Jeon JY, Talebi Z, *et al.* Gilteritinib-induced upregulation of S100A9 is mediated through BCL6 in Acute Myeloid Leukemia. Blood Adv 2021. [Epub ahead of print]

146. ▪ Duy C, Li M, Teater M, *et al.* Chemotherapy induces senescence-like resilient cells capable of initiating aml recurrence. Cancer Discov 2021; 11:1542–1561.

Anthracyclines induces a subpopulation of leukemic cells to acquire an inflammatory signature and senescence-like state that contributes to chemoresistance.

147. Degorce SL, Tavana O, Banks E, *et al.* Discovery of proteolysis-targeting chimera molecules that selectively degrade the IRAK3 pseudokinase. J Med Chem 2020; 63:10460–10473.

148. Zhang Y, Diao N, Lee CK, *et al.* Neutrophils deficient in innate suppressor IRAK-M enhances antitumor immune responses. Mol Ther 2019; 28: 89–99.

