



## Revisiting the Role of TLR/IRAK Signaling and its Therapeutic Potential in Cancer

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### The Disappointment of TLR Agonists as Cancer Immunotherapeutic Drug

Interleukin-1 receptor-associated kinase (IRAK) family consists of four members: IRAK1, IRAK2, IRAK3/M and IRAK4. They are key downstream mediators of toll-like receptor (TLR) and interleukin-1 receptor (IL1R) pathways. In particular, TLR, a family of transmembrane pattern recognition receptor (PRR), play a crucial role in the induction of innate and adaptive immune responses against microbial infection, tissue injury and also cancer. As PRR, the 11 members of TLR family can each recognize distinct pathogen-associated molecular patterns (PAMPs) [e.g. lipopolysaccharide (LPS), flagellin] or damage-associated molecular patterns (DAMPs) [e.g. high-mobility group box 1 (HMGB1), DNA and RNA], and initiate immune responses via IRAK signaling. Upon binding of PAMPs or DAMPs to TLRs, myeloid differentiation primary response protein 88 (MyD88) adaptor proteins is recruited to TLRs via toll-interleukin-1 receptor (TIR) domain on both molecules. MyD88 then associates with IRAK4 and IRAK2 to form the MyD88/IRAK4/IRAK2 Myddosome complex, which activate IRAK1 through IRAK4-dependent phosphorylation. Activated IRAK1 then detached from the complex and binds the E3 ubiquitin ligase tumor necrosis factor receptor (TNFR) associated factor 6 (TRAF6), which lead to the activation of two distinct pathways: activator protein 1 (AP-1) transcription factor thorough activation of MAP kinases and NF- $\kappa$ B transcription factor through activation of I $\kappa$ B kinase (IKK) complex. On the other hand, the negative regulator IRAK3/M works by inhibiting the dissociation of Myddosome and thus preventing the activation of downstream signaling.

Naturally, in concordance with their immune-surveillance role, TLRs are predominantly expressed on immune defense related tissues (e.g. spleen, peripheral blood leukocytes) or those that encounter external environment (e.g. gastrointestinal tract, liver and lung) [1]. Engagement of TLR initiates rapid activation of immune cells including natural killer (NK) cells, dendritic cells (DC) and macrophages; and induction of a range of pro-inflammatory cytokines including IFN- $\alpha$ , IFN- $\gamma$ , TNF- $\alpha$ , IL-1, IL-6 and IL-12. Furthermore, TLRs is imperative for T cells responses through TLR-mediated activation of antigen presenting cells (APCs), particularly DCs, by augmenting their expression of costimulatory molecules

such as CD155 and antigen-presenting activity [2]. The potential of TLRs activation to boost anti-tumor immune response and hamper immune tolerance has made it an attractive target for the development of cancer immunotherapy. The history of TLR pathway targeted therapy actually dates back to 1890s when Dr. William Coley observed spontaneous tumor regression in cancer patients who experienced post-surgical infections [3]. Coley's toxins were later developed based on the idea that microbial products could provoke antitumor immune responses and leads to tumor regression [3]. This forms the basis of today's anticancer TLR agonist immunotherapy. TLR agonists were first introduced with high hope as immunotherapeutic agents to treat cancer and several TLR agonists have gone on to clinical trials [4]. However, despite promising results from research studies, TLR agonists continue to disappoint as clinical drugs. The most notable downfall was the ending of the Phase III clinical trial of TLR9 agonist ProMune by Pfizer, which did not improve survival of non-small-cell lung cancer patients but rather increased toxicity [5].

### Chronic Up-Regulation of TLR/IRAK Signaling in Cancer Promote Carcinogenesis

One ought to wonder why TLR agonists, once praised as the highest potential immunotherapy drug by the US National Cancer Institute, did not suffice its purpose. Past researches had centered on harnessing the TLR/IRAK signaling to boost anti-cancer immune response. However, the consequences of aberrant up-regulation of the pathway were overlooked. TLR/IRAK pathway may promote tumorigenesis by providing a favorable microenvironment for cancer to thrive. For instance, by sustaining chronic inflammation or production of cytokines or chemokines that promotes cell proliferation and survival. It is long established that chronic inflammation predisposes to and sustain carcinogenesis, and represents a characteristic tumor micro environment [6]. Many cancers emerges from sites of chronic inflammation, such as hepatitis C virus infection-induced hepatitis leads to Hepatocellular Carcinoma (HCC); inflammatory bowel disease leads to colon cancers and Helicobacter pylori-induced gastritis to gastric cancer. Taking HCC as an example, hepatitis induced by viral infection will initiate an immune response due to PAMPs (from viral infection) and DAMPs (from damaged cells), characterized by infiltration of immune cells including macrophages, DCs, NK cells, T and B-lymphocytes. This immune response contributes to viral clearance and tumor elimination at early stage [7]. However, chronic up-regulation of the TLR/IRAK pathway could leads to production of excessive pro-inflammatory mediators (e.g. IL-6, IL-1 $\beta$ , TNF- $\alpha$ ) through the downstream NF- $\kappa$ B signaling and promote hepatic fibrosis and cirrhosis, which strongly enhance the risk of hepatocellular carcinoma (HCC) [7]. Furthermore, DAMPs from stressed or dying hepatocytes provide further feed-forward signals to support this hepatotoxic cycle of inflammation and cell deaths, favoring cancer development [7].

Alternatively, TLR/IRAK up-regulation could promote carcinogenesis by directly affecting the tumor cells. TLRs were once thought to only express on immune cells. While there is no doubt that immune cells express the most diverse sets of TLRs, accumulating evidences point out TLRs are also widely expressed on various cancer cells [8]. Moreover, over-activation of the downstream IRAK

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signaling in cancer has been reported in several studies [9], indicating TLR/IRAK signaling play an imperative role in cancer development. One route is to enhance the survival signals in cancer cells through TLR/IRAK mediated activation of NF- $\kappa$ B signaling, a central regulator of anti-apoptotic genes expression such as Bcl-2 and Bcl-XL and pro-apoptotic pathways activation [10]. Another route is to allow cancer cells to evade immunosurveillance and escape attack from cytotoxic T lymphocytes, through production of inhibitory cytokines and down regulation of Fas expression in TLR4 overexpressing cancers [11]. In addition, TLR/IRAK signaling is shown to enhance tumor angiogenesis, promote tumor growth as well as contributing to chemo resistance in various cancers [12].

### The Emergence of IRAK1/4 Inhibitor as a Novel Therapeutic Drug

In spite of the increasing evidences on the tumor-promoting effects of TLR/IRAK signaling, recent research direction has been progressively shifted to focus on TLR antagonist for cancer treatment, or more specifically, the downstream IRAK signaling that converge signals from all TLRs activation (except TLR3). The carcinogenic effect of aberrant IRAK signaling first came into light in 2011, when a gain of function mutation at L265P of MyD88 was discovered [13]. This mutation leads to spontaneous IRAK4-dependent phosphorylation of IRAK1 and thus constitutive up-regulation of NF- $\kappa$ B and JAK kinase signaling, eventually promoting the survival of malignant cell in activated B-cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL) [13]. Knockdown of MyD88, IRAK4 or IRAK1 in L265P mutation-bearing ABC DLBCL cell lines abrogated NF- $\kappa$ B activation and leads to rapid apoptosis [13]. The authors lastly proposed the development of IRAK4 inhibitor to treat cancer mediated by MyD88 signaling [13]. In fact, development of IRAK specific inhibitor dates back to 2006 when Powers et.al., first discovered a novel series of N-acyl 2-aminobenzimidazoles that specifically target IRAK family [14]. This discovery allows later refinement and development of more potent IRAK4 or IRAK1/4 inhibitor. While all four members of IRAK family bear non-redundant biological functions, only IRAK1 and IRAK4 exhibit serine/threonine kinase activity and are therefore targetable [9]. Please note there is no IRAK1 specific inhibitor yet as its crystal structure remains to be elucidated.

The therapeutic potentials of IRAK inhibitors were first focused on treating autoimmune and inflammatory diseases such as sepsis, asthma and rheumatoid arthritis. But since the emerging evidences linking TLR/IRAK signaling to cancer development, it has been increasingly used in cancer study. To date, the use IRAK1/4 inhibitor has demonstrated promising anti-cancer results in melanoma [15], myelodysplastic syndrome (MDS) [16] and T cell acute lymphoblastic leukemia (T-ALL) [17]. In regards to melanoma, the authors showed that combined treatment of IRAK1/4 inhibitor with vinblastine augmented apoptotic responses *in vitro* and *in vivo* and improved survival when used to treat mice with established melanoma tumor [15]. In the MDS study, the authors identified IRAK1 overexpression and hyper-activation is a common phenomenon in MDS [16]. They further demonstrated that either genetic ablation of IRAK1 or the use of IRAK1/4 inhibitor induced apoptosis and cell-cycle arrest in MDS [16]. Most importantly, both pre-treatment of the MDS-derived patient cell line (MDSL) cells before transplanted into mice or administration of the inhibitor after xenotransplantation of MDSL cells resulted in prolonged survival and the effect was augmented when combined with bcl-2 inhibitor ABT-263 [16]. Lastly, in the most recent T-ALL study, the authors showed T-ALL

cells from patients have overexpression of IRAK1 and IRAK4 in both mRNA and protein level (both total and phosphorylated forms) [17]. Knockdown of IRAK1 or IRAK4 by either genetic approach or IRAK1/4 inhibitor approach significantly impaired proliferation of T-ALL cells in *in vitro* and *in vivo* models, while showing minimal apoptotic effect [17]. Furthermore, they identified IRAK1/4 inhibitor when combined with either ABT-737 or vincristine augmented therapeutic responses, reduced in T-ALL cell numbers and prolonged survival in a mice leukemia model [17].

### Final remarks

In conclusion, the above studies demonstrated the huge potentials of IRAK1/4 in cancer therapeutic targeting, mainly through augmentation of the efficacy of chemotherapeutic drugs and dampening the tumor promoting effect of the TLR/IRAK pathway. Nevertheless, it is important to note that due to the diverse types of TLRs and their varied expression on different cell types, TLR/IRAK activation could result in different responses in different contexts, depending on the disease stage, tumor type and also surrounding cells. And the final response to TLRs stimulus represents the orchestra between the immune cells, stromal cells and tumor cells within the tumor microenvironment. Therefore it is of particular importance to fully characterize the effect of therapeutically targeting the TLR/IRAK pathway in different cancer models. Another concern is that inhibiting the TLR/IRAK pathway with inhibitors may hamper the anti-cancer immune response initiated by this pathway; therefore alternative ways can be used to preserve this immune response. For instance by combining IRAK1/4 inhibitor with neutralizing antibody against CD47 [18] or explore adoptive cell therapy of *ex vivo* activated and expanded T cell or NK cells to activate the anti-cancer immune response [19].

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