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New horizons in adjuvants for vaccine development
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Adjuvant molecules, particularly toll like receptor (TLR) agonists have been in development for decades, though until now only a natural TLR 4 ligand (mono-phosphoryl lipid A, MPL) has been incorporated into licensed vaccine products, in formulations that enhance and complement the MPL activity. The inclusion of MPL-based formulations into vaccines has been based on enhancing antibody responses to subunit antigens, and has provided important proof-of-concept for enhancing desired immune responses to defined molecular targets. Challenges remain in adjuvant development, particularly for those that stimulated effective T cell responses for both preventative and therapeutic vaccines. The discovery of molecules, many based on RNA, that stimulate innate and adaptive immune responses and have the ability to stimulate potent CD8 T cell responses, has opened the door for development of a new generation of vaccines.

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Introduction
With the rapid developments around diseases caused by coronaviruses, understanding and applying knowledge relevant to inducing potent anti-viral responses become increasingly critical. The focus of this discussion is on molecules discovered based on anti-viral activity and the potential for using these formulated molecules as vaccine adjuvants. Adjuvants are critical components for developing effective subunit vaccines. Over the past decades, formulations of the glycolipid monophosphoryl lipid A, or MPL [1], have been incorporated into vaccines targeting viral, bacterial, and parasite pathogens with varying degrees of success, including several with regulatory approvals. The mammalian immune system recognizes pathogen — associated molecular patterns (PAMPs) via pathogen recognition receptors (PRRs) that include toll-like receptors (TLR) as well as other categories of receptors classified as (NOD) or retinoic acid inducible gene-I (RIG-I) and the RIG-I-like receptors (RLRs) [1]. MPL, the most advanced TLR-4 agonist, has been used to develop combination adjuvants, either with alum (AS04) or with QS-21, a saponin derivative (AS01), that are now in licensed products [2,3]. The most recent and best example of a critical role for adjuvants for vaccine efficacy is with AS01-containing Shingrix, the shingles vaccine with >90% efficacy. It is widely recognized that MPL and subsequent TLR-4 ligands, including glycopeptidolipid A (GLA) and the related second generation molecule SLA, can induce potent Th1 biased immune responses with corresponding high quality and durable antibodies, also demonstrated with DNA-based adjuvants, including the ISS (immunostimulatory sequences, TLR-9 activating CpG) used in the HEPL-Slav Hep B vaccine [4]. Development through licensure of products containing MPL-based and CpG-based adjuvants has been a remarkable success. In both cases, first licensures have been for prophylactic vaccines against hepatitis B, in which unmet needs in terms of dosage sparing (i.e. fewer injections needed to reach approvable endpoints) as well as increased responsiveness in elderly/immune compromised groups were demonstrated. Less developed are adjuvants that activate via other PRR, including those that stimulate via TLR-3, TLR-7, TLR-8, or TLR-7/8, or RIG-I. Each of these pathways can be activated with RNA, and a variety of synthetic agonists that function via these pathways have been synthesized and formulated. The excitement around the potent adjuvant activity of 3M-052, a TLR 7/8 agonist, and the RNA-based adjuvants activating TLR-3 and RIG-I pathways has come from data generated with a variety of vaccine candidates that suggest the ability to expand upon the depth of immune responses obtained thus far using TLR-4 based adjuvants, including CD8 responses.

TLR7 and TLR8 agonists
TLR7 and TLR8 are expressed intracellularly in different cells with human plasmacytoid dendritic cells, T helper cells and B cells expressing TLR7, while TLR8 is expressed on conventional dendritic cells, monocytes and macrophages and regulatory T cells [5,6]. Because TLR7 and TLR8 are expressed on different immune cells these agents stimulate different cytokine profiles and may, therefore, stimulate the adaptive immune response differently. Stimulation of TLR7 and TLR8 with small molecules and ssRNA enhances DC activation as well as activation both humoral and cell-mediated immunity. A number of companies are developing similar
TLR7 and TLR8 agonists as vaccine adjuvants including 3M, GSK, and VentiRx.

First generation TLR 7/8 agonists like the TLR7 agonist imiquimod, initially developed as an anti-viral, and the TLR7/8 agonist resiquimod have shown potent adjuvant activity in a number of animal models [5]. Despite this benefit the adjuvant effects were only seen at concentrations that induced systemic cytokines which lead to systemic side-effects. TLR 7/8 agonists that induce local adjuvant effects without inducing systemic cytokines should not only be more efficacious as vaccine adjuvants but also less toxic.

To that end 3M has developed a novel TLR7/8 agonist 3M-052 that has a fatty acid tail added to the 1-position of the imidazoquinoline ring which allows the molecule to stay at the vaccination site when injected as part of typical vaccine formulations [7]. Formulations of 3M-052 showed potent adjuvant effects in combination with H1N1, hepatitis B surface antigen and amebiasis vaccines at doses that induced no systemic TNF-α [7–9]. Studies by Fox et al. demonstrated that appropriate formulation of 3M-052 was important for optimizing the immune response [10]. Additional studies in non-human primates showed potent adjuvant effects of 3M-052 in combination with HIV and pneumococcal vaccines in adult and neonatal animals [11,12]. When looking at neonatal NHPs immunized with HIV env protein, 3M-052 increased IgG responses above the ALUM control and the animals immunized with a TLR4 agonist. In addition, 3M-052 was the only agonist to induce cross clade IgG responses [12*]. Doses that were used in these studies were well tolerated and showed limited systemic side effects and local toxicity.

Pharmacokinetic studies demonstrated that subcutaneous (s.c.) or intramuscular (i.m.) injection of 3M-052 lead to only low levels of the parent compound in the serum as compared to results seen with resiquimod (Tomai, personal observation). Unlike resiquimod, 3M-052 did not induce systemic cytokines, even when dosed at 1 mg/kg, and 3M-052 demonstrated superior antigen sparing activity. Taking into account these positive results, 3M-052 was evaluated for toxicity in rats in combination with an H7N1 vaccine. Results demonstrated that 3M-052 was well tolerated at the doses evaluated. Recently, two studies have demonstrated that formulated 3M-052 was a potent adjuvant for inducing effective B and T cell responses in non-human primates in the context of HIV antigens [13,14]. Taken together, these results support further development of 3M-052 for use in human clinical studies.

There are other TLR7 agonists being developed as vaccine adjuvants. A benzonaphthyridine TLR7 agonist containing a phosphonate group is capable of complexing to Alum and has been shown to be capable of enhancing responses to various glycoconjugate vaccines and a vaccine to Bordetella pertussis [15,16]. In the Bordetella model the TLR7 adjuvant response was as effective as the whole cell vaccine. Mechanistically the TLR7 adjuvant enhanced switching of the antibody response towards IgG2a/b and induced T cell production of IL-17 and IFN-gamma. The TLR7 agonist adsorbed to ALUM also boosted vaccine responses to staphylococcus aureus and RSV 2 pathogens where there is no effective vaccine [6].

On the TLR8 side, bacterial RNA and small molecules that activate through TLR8 activate conventional DC and specifically enhanced T follicular cell responses and may act as potent adjuvants for cell-mediated immune responses [6]. Messenger-RNA has also been shown to have potent adjuvant activity in an influenza model that is in part mediated by TLR7 and TLR8 [11]. In summary, adjuvants containing TLR7 and 8 can act as potent adjuvants of both humoral and cell-mediated immune responses.

**TLR3 agonists**

Among the first data demonstrating the potential of using RNA were generated using poly-ICLC, or poly (I:C). A wealth of promising data, both clinical and pre-clinical, have been generated with poly-ICLC as well as with other TLR-3 agonists, though development of approved products containing this class of adjuvants has yet to be realized. Poly-ICLC (Hiltonol, Oncovir, Inc.) is a synthetic double stranded RNA that binds to TLR-3, MDA-5, among others, leading to effective activation of dendritic and NK cells [16]. Interestingly, there is evidence for synergy between TLR-3 and RIG-I innate activation pathways, each stimulated by RNA [17].

While the scientific rationale for development of TLR-3 agonist is apparent due to advantages of cellular activities induced and the resulting immune responses, there have been issues that have hindered advanced development. These include problems with consistent manufacturing of the RNA-based TLR3 agonists. Improvements in agonist design and manufacturing have led to the development of Riboxol, a 50 base pair RNA developed by Riboxx. Advantages of Riboxol and the 100 base pair precursor, RGC100 [18] include molecular homogeneity, and manufacturing advantages. Riboxol appears to bind exclusively to TLR-3, thus it may differ mechanistically from earlier versions of TLR-3 agonists.

When properly formulated, an important issue that has been long overlooked, Riboxol and related next generation molecules have tremendous potential as next generation adjuvants. RNA-based adjuvants, for example, TLR-3 and RIG-I agonists, exemplify the importance of formulations that can both protect the RNA molecules from degradation, as well as increase efficient intracellular
uptake by antigen presenting cells for activating the endosomal TLR-3 receptor. Safe and effective nanoformulations have been developed and extensively used in the clinic, the next step is to combine them with TLR-3 ligands, among others, to develop effective adjuvants and therapeutics.

Activation of the TLR-3 pathway appears to be effective in inducing a broad range of immune responses, including CD8 responses, in humans and non-human primates [19–22]. Although TLR-3 agonists have great potential as vaccine adjuvants, much of the clinical data generated with poly-ICLC has been in cancer therapy. Rationale for treating solid tumors with TLR ligands has come from several clinical studies, mostly with poly-ICLC but also with 3M052 and the GLA-SE TLR4 agonist. and have demonstrated the potential of generating both innate responses leading to tumor shrinkage, but also adaptive responses to essentially turn the tumor into a vaccine [23,24].

RIG-I
RIG-I is the charter member of the RIG-I-like receptor (RLR) family of RNA helicases that includes RIG-I, melanoma differentiation antigen (MDA)5, and Laboratory of Genetics and Physiology (LGP2) proteins. The RLRs are expressed at a low level in most if not all cell types and tissues, and their levels are increased in response to various cytokines including type 1 and type 3 interferon (IFN) [25]. RIG-I and MDA5 function as essential PRRs for the primary recognition of RNA virus infection whereas LGP2 is less-well understood but is thought to regulate RIG-I and MDA5 function [26]. The RLRs are cytosolic proteins belonging to the RNA helicase superfamily. In response to RNA virus infection, the RLRs recognize and bind to nonsense signature motifs that constitute pathogen associated molecular pattern (PAMP) within viral RNA replication products. In response to PAMP binding RIG-I hydrolyze ATP and undergo a confirmation change that permits interaction with cofactors including tripartite motif 25 (TRIM25) and 14-3-3 epsilon (14-3-3ε) προτεινων. TRIM25 mediates RIG-I ubiquitination that facilitates signaling activity [27] while 14-3-3ε chaperones the translocation of RIG-I from the cytosol to mitochondria-associated membranes for binding to the mitochondria antiviral signaling (MAVS) adaptor protein [28]. MAVS binding by RIG-I or MDA5 then activates the MAVS signalosome comprises tumor necrosis factor receptor associated factor (TRAF) proteins, interferon regulatory factor (IRF)3/7, and the NF-kB kinases and other signaling partners to impart downstream transcription factor activation including IRF3, IRF7, and NF-kB leading to innate immune activation and the expression of immune regulatory genes [25] (PMID). RIG-I has also been shown to direct the activation of activating transcription factor (ATF)4 and SMAD4 (abbreviation is from the combination of SMA (‘small’ worm phenotype) and Drosophila MAD (‘Mothers Against Decapentaplegic’) family of genes), impacting immune polarization and the inflammatory response [29].

While many studies have shown that RIG-I is essential for triggering innate immunity and for proper immune polarization against infection by RNA viruses [30–32], recent work reveals RIG-I as an attractive target for immune programming for vaccine adjuvant actions, directing antiviral innate immunity, and as an adjuvant to mediate cancer immunotherapy. RIG-I recognizes and binds to PAMP RNA motifs marked by a free 5’ triphosphate (5’ppp), double stranded (ds)RNA structure, and poly-uridine signature [33]. Synthetic PAMP RNA motifs have been produced and used in preclinical studies to evaluate the immune regulatory actions of activating RIG-I with therapeutic PAMP RNA [33]. These studies show that targeting RIG-I with PAMP RNA can facilitate local innate immune activation for restriction of virus infection, enhancement of vaccine efficacy, and for immune-oncology enhancement of tumor suppression [34]. Targeting of RIG-I to activate innate immunity via treatment of cultured cells with PAMP RNA or small molecule agonists of RIG-I resulted in robust innate immune activation and suppression of infection by a broad range of viruses [35*,36]. Moreover, small molecule agonists of RIG-I exhibit potent adjuvant actions to enhance antiviral immunity from influenza A virus split vaccine in vivo [37]. Treatment of mice with 5’ppp-RNA engineered to optimally activate RIG-I leads to induction of local and intra-tumoral innate immunity with enhancement of cell-mediated immunity against a variety of tumor types, resulting in tumor shrinkage and cancer remission [38]. RIG-I activation was shown to impart 5’ppp- RNA adjuvant actions to control the growth of a variety of cancer cell types, revealing RIG-I as a key target for immune-oncology applications of cancer therapy [39]. Moreover, activation of RIG-I is also shown to direct the germlinal center reaction to enhance high quality antibody production [40] (PMID). Overall, these studies define the utility of RLR targeting for therapeutic applications to activate innate immunity and enhance cell and humoral adaptive immune responses for the control of virus infection, enhancement of vaccine protection, and to facilitate tumor control.

Conclusions
Several review articles have described the variety of ligands for TLR and related innate immune pathways [41]. Progress has been made in the development of agonist molecules and characterization of the molecular activation pathways they activate, and more recently, in the ability to formulate agonists to enable safe and effective in vivo applications. Formulations appropriate for human vaccines include alum, oil/water emulsions (e.g. MF59), and liposomes. The necessity of formulation
is particularly important for delivery of RNA-based agonists of TLR-3 and RIG-I, both for protection of molecules from RNA-se activity and, in the case of TLR-3, delivery to endosomal receptors. In spite of real progress, few adjuvants have made their way into approved vaccines and therapeutics, though the examples of Shingrix and HEPLISAV have done much to reduce barriers by demonstrating solutions to a previously unmet need. Challenges remain, including those of scaled manufacturing. However, these issues are also being addressed by advances in developing optimized synthetic processes to improve existing products or to replace natural products (e.g., TLR-3, TLR-4 agonists) as well as by optimizing formulations increase efficiency of delivery and thus allow for reducing the dose of agonist needed for an effective immune response. The potential of RNA-based and small molecule adjuvant formulations, particularly targeting RIG-I for activating innate immunity and enhancing CD8 and antibody responses adds impetus to the field of adjuvant development for both vaccines and therapeutics.

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Conflict of interest statement
Mike Gale is an inventor of the RIG I technology and academic founder of HDT Bio.

Mark Tomai is an inventor of 3M-052 and is employed by 3M Corporation.

Steven Reed is Founder and CEO of HDT Bio, which has license rights to RIG-I and TLR-3 technologies.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest

1. Kawasaki T, Kawai T: Toll-like receptor signaling pathways. Front Immunol 2014, 5:461 PMID 25309543.

2. Fox CB et al.: Current status of TLR4 ligand vaccine adjuvants. In: Immunopotentiators in Modern Vaccines. Edited by O’Hagan DT, Schijns V. Elsevier; 2017:105-128.

3. Bharucha T et al.: A critical appraisal of “Shingrix”, a novel herpes zoster subunit vaccine (HZ/Su or GSK 1437173A) for varicella zoster virus. Hum Vaccine Immunother 2017, 13:1789-1797 PMID 28426274.

4. Nelson FE et al.: The potential of 1018 ISS adjuvant in hepatitis B vaccines. HEPLISAV review. Human Vaccine Immunther 2013, 9:1661-1672 PMID 23732907.

5. Vasilakos JP, Tomai MA: The use of Toll-like receptor 7/8 agonists as vaccine adjuvants. Expert Rev Vaccines 2013, 12:809-819.

6. Dowling DJ: Recent advances in the discovery and delivery of TLR7/8 agonists as vaccine adjuvants. ImmunoHorizons 2018, 2:185-197.

7. Smirnov D, Schmidt JJ, Capecchi JT, Wightman PD: Vaccine adjuvant activity of 3M-052: an imidazolinequinolone designed for local activity without systemic cytokine induction. Vaccine 2011, 29:5434-5442.

8. Abhyankar MM, Orr MT, Lin S, Suraju MO, Simpson A, Blust M, Pham T, Guderian JA, Tomai MA, Elvecrog J et al.: Adjuvant composition and delivery route shape immune response quality and protective efficacy of a recombinant vaccine for Entamoeba histolytica. NPJ Vaccines 2018, 3:22.

9. Van Hoeven N, Fox CB, Granger B, Evers T, Joshi SW, Nana GI, Evans SC, Lin S, Liang H, Liang L, Nakajima R et al.: A formulated TLR7/8 agonist is a flexible, highly potent and effective adjuvant for pandemic influenza vaccines. Sci Rep 2017, 7:46426.

10. Fox CB, Orr MT, Van Hoeven N, Parker SC, Mikasa T, Phan T, Beeke EA, Nana GI, Joshi SW, Tomai MA et al.: Adsorption of a synthetic TLR7/8 ligand to aluminum oxyhydroxide for enhanced vaccine adjuvant activity: a formulation approach. J Control Release 2016, 244:98-107.

11. Dowling DJ, van Haren SD, Scheid A, Bergelson I, Kim D, Mancuso CJ, Foppens W, Ozonoff A, Fresh L, Theriot TB et al.: TLR7/8 adjuvant overcomes newborn hyporesponsiveness to pneumococcal conjugate vaccine at birth. JCI Insight 2017, 2:e91029.

12. Phillips B, Van Rompay KKA, Rodriguez-Nieves J, Lorin C, Koutsoukos M, Tomai M, Fox CB, Eudaley J, Dennis M, Alam SM et al.: Adjuvant-dependent enhancement of HIV Env-specific antibody responses in infant rhesus macaques. J Virol 2018, 92 Infant macaques immunized in a prime and boost setting with HIV envelope proteins in combination with a stable emulsion formulation of the TLR7/8 agonist 3M-052 enhanced antibody responses more effectively than Alum or the TLR4 agonist GLA that was also formulated in an emulsion. The breadth of the antibody response as measured by cross-clade protection was enhanced most effectively by 3M-052 SE. The avidity of the antibody response and the ability of the antibody to induce antibody dependent cellular cytotoxicity were increased most effectively by the 3M-052 SE adjuvant.

13. Arunachalam PS et al.: T cell-inducing vaccine durably prevents mucosal SHIV infection even with lower neutralizing antibody titers. Nat Med 2020 http://dx.doi.org/10.1038/s41591-020-0858-8.

14. Kasturi SP et al.: 3M-052, a synthetic TLR7/8 agonist, induces durable HIV-1 envelope-specific plasma cells and humoral immunity in nonhuman primates. Sci Immunol 2020, 5 eabb 1025.

15. Edwards DK, Jasny E, Yoon H, Horstcroft N, Schanen B, Geter T, Fotin-Mleezck M, Petch B, Wittman V: Adjuvant effects of a sequence-engineered mRNA vaccine: translational profiling demonstrates similar human and murine innate response. J Transl Med 2017, 15:1.

16. Alexopoulou L et al.: Recognition of double-stranded RNA and recognition of NF-kappa B by toll like receptor 3. Nature 2001, 413:732-738.

17. Palm MW, Medzhitov R: Pattern recognition receptors and control of adaptive immunity. Immunol Rev 2009, 227:221-233.

18. Naumann K et al.: Activation of dendritic cells by the novel TLR-3 agonist RGC100. Clin Dev Immunol 2013, 2013 283649.

19. Caskey M et al.: Synthetic double stranded RNA induces innate immune responses similar to a live viral vaccine in humans. J Exp Med 2011, 208:2357-2366.

20. Stahl-Hennig C et al.: Synthetic double stranded RNAs are adjuvants for induction of T helper 1 and humoral immune responses to human papilloma virus in rhesus macaques. PLoS Pathog 2009, 5:e1000037 PMID:PMC2660151.

21. Tewari K et al.: Poly(I:C) is an effective adjuvant for antibody and multi-functional T cell CD4 responses to Plasmodium falciparum circumsporozoite protein (CSP) and sDEC CSP in Non-Human Primates. Vaccine 2010, 28:7256-7266.

22. Okada et al.: Induction of robust type-1 CD8+ T cell responses in WHO grade II low-grade glioma patients receiving peptide-
based vaccines in combination with poly-I:C. Clin Cancer Res 2016, 10 1158-1078-0432.

23. Temizoz B et al.: Vaccine adjuvants as potential cancer immunotherapeutics. Nat Immunol 2016, 28:329-338 PMID 2706304.

24. Kyi C et al.: Therapeutic immune against solid cancers with Poly-I:C-LC: a pilot trial. Clin Cancer Res 2018, 24:4937-4948 PMID29950349.

25. Chow KT, Gale M Jr. Looy YM: RIG-I and other RNA sensors in antiviral immunity. Annu Rev Immunol 2018, 36:667-694 http://dx.doi.org/10.1146/annurev-immunol-042617-053309.

26. Looy YM, Gale M Jr: Immune signaling by RIG-I-like receptors. Immunity 2011, 34:680-692 http://dx.doi.org/10.1016/j.immuni.2011.05.003.

27. Gack MU, Shin YC, Joo CH, Urano T, Liang C, Sun L, Takeuchi O, Akira S, Chen Z, Inoue S, Jung JU: TRIM25 RING-finger 3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. Nature 2007, 446:916-920.

28. Liu HM, Looy YM, Homer SM, Zornetzer GA, Katze MG, Gale M Jr: The mitochondrial targeting chaperone 14-3-3σ regulates a RIG-I translocon that mediates membrane association and innate antiviral immunity. Cell Host Microbe 2012, 11:528-537 http://dx.doi.org/10.1016/j.chom.2012.04.006.

29. Stone AEL, Green R, Wilkins C, Hemann EA, Gale M Jr: RIG-I-like receptors direct immunomodulatory macrophage polarization against West Nile virus infection. Nat Commun 2019, 10:3649-3663 http://dx.doi.org/10.1038/s41467-019-11250-5.

30. Sprokholt JK, Kaptein TM, van Hamme JL, Overmars RJ, Gringhuis SI, Geijtenbeek TBH: RIG-I-like receptor activation by dengue virus drives follicular T helper cell formation and antibody production. PLoS Pathog 2017, 13:e1006738-1006745 http://dx.doi.org/10.1371/journal.ppat.1006738 eCollection.

31. Sprokholt JK, Kaptein TM, van Hamme JL, Overmars RJ, Gringhuis SI, Geijtenbeek TBH: RIG-I-like receptor triggering by dengue virus drives dendritic cell immune activation and TH1 differentiation. J Immunol 2017, 198:4764-4771 http://dx.doi.org/10.4049/immunol.1602121 Epub 2017 May 15.

32. Fournier P, Arnold A, Schirmacher V: Polarization of human monocyte-derived dendritic cells to DC1 by in vitro stimulation with Newcastle Disease Virus. J BUON 2000, 14(Suppl. 1):S111-S122.

33. Kell AM, Gale M Jr: RIG-I in RNA virus recognition. Virology 2015, 479:10-121 http://dx.doi.org/10.1016/j.virology.2015.02.017 Epub 2015 Mar 5.

34. O’Donovan DH, Mao Y, Mele DA: The next generation of pattern recognition receptor agonists: improving response rates in cancer immunotherapy. Curr Med Chem 2019, 10:2174-2184 Epub ahead of print.

35. Schnell G, Looy YM, Marcotrigiano J, Gale M Jr: Urudine • composition of the poly-U/UC tracts of HCV RNA defines non-self recognition by RIG-I. PLoS Pathog 2012, 8:e1002839- e1002852 http://dx.doi.org/10.1371/journal.ppat.1002839 Epub 2012 Aug 2

This study presents a structure-function analysis of the RIG-I ligand pathogen associated molecular pattern (PAMP) motif of the hepatitis C virus genome. The ideal PAMP motif was defined as a 5’ triphosphate poly-uridine cytosine RNA with flanking c/g dinucleotide motif-containing 5’ and 3’ arms, denoted as pU/UC PAMP. RIG-I specifically recognizes and binds to the pU/UC PAMP. The pU/UC PAMP activates the RLR pathway in vivo and has therapeutic antiviral properties.

36. Pattabhi S, Wilkins CR, Dong R, Knoll ML, Posakony J, Kaiser S, Mire CE, Wang ML, Iretton RC, Geisbert TW et al.: Targeting innate immunity for antiviral therapy through small molecule agonists of the RLR pathway. J Virol 2013, 90:2372-2387 http://dx.doi.org/10.1128/JVI.02002-15.

37. Probst P, Grigg JB, Wang M, Muñoz E, Looy YM, Iretton RC, Gale M Jr, Iadonato SP, Bedard KM: A small-molecule IFR3 agonist functions as an influenza vaccine adjuvant by modulating the antiviral immune response. Vaccine 2017, 35:1964-1971 http://dx.doi.org/10.1016/j.vaccine.2017.01.053 Epub 2017 Mar 6

This study assessed the signaling and adjuvant activities of a small molecule (SM) RIG-1 agonist to enhance influenza A virus vaccine efficacy in a mouse model in vivo. SM RIG-1 agonist activates downstream interferon regulatory factor-3 signaling and the induction of IFR3-target genes that program the immune response. SM RIG-1 agonists activate RIG-I to drive a response that enhances the production of high level neutralizing antibodies induced by a influenza A virus split vaccine. Targeting RIG-I has potent immune adjuvant activity.

38. Duewell P, Steger A, Lohr H, Bourhis H, Hoelz H, Kirchleitner S, Stieg MR, Grassmann S, Kobold S, Siveke JT et al.: RIG-I-like helicases induce immunogenic cell death of pancreatic cancer cells and sensitize tumors toward killing by CD8(+)/T cells. Cell Death Differ 2014, 21:1825-1837 http://dx.doi.org/10.1038/cdd.2014.96 Epub 2014 Jul 11.

39. Elion DL, Cook RS: Harnessing RIG-I and intrinsic immunity in the tumor microenvironment for therapeutic cancer treatment. OncoTarget 2018, 9:29007-29017 http://dx.doi.org/10.18632/oncotarget.25626 eCollection 2018 Jun 22.

40. Kulkarni RR, Rasheed MA, Bhaumik SK, Ranjan P, Cao W, Davis C, Marietti K, Thomas S, Gangappa S, Sambhara S, Murali-Krishna K: Activation of the RIG-I pathway during influenza vaccination enhances the germinal center reaction, promotes T follicular helper cell induction, and provides a dose-sparing effect and protective immunity. J Virol 2014, 88:13990-134001 http://dx.doi.org/10.1128/JVI.02273-14 Epub 2014 Sep 24.

41. Duthie M et al.: Use of defined TLR ligands as adjuvants within human vaccines. Immunol Rev 2011, 239:178-196.