Trial watch: TLR3 agonists in cancer therapy

Julie Le Naour, Lorenzo Galluzi, Laurence Zitvogel, Guido Kroemer, Erika Vacchelli

OncoImmunology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/koni20

Trial watch: TLR3 agonists in cancer therapy

Julie Le Naour , Lorenzo Galluzzi , Laurence Zitvogel , Guido Kroemer & Erika Vacchelli

To cite this article: Julie Le Naour , Lorenzo Galluzzi , Laurence Zitvogel , Guido Kroemer & Erika Vacchelli (2020) Trial watch: TLR3 agonists in cancer therapy, OncoImmunology, 9:1, 1771143, DOI: 10.1080/2162402X.2020.1771143

To link to this article: https://doi.org/10.1080/2162402X.2020.1771143

Published online: 02 Jun 2020.

ONCOIMMUNOLOGY
2020, VOL. 9, NO. 1, 1–13
https://doi.org/10.1080/2162402X.2020.1771143

Taylor & Francis
Taylor & Francis Group

REVIEW

Trial watch: TLR3 agonists in cancer therapy

Julie Le Naour[a,b,c,d], Lorenzo Galluzzi[e,f,g,h,i], Laurence Zitvogel[c,j,k], Guido Kroemer[a,b,c,k,l,m,n*], and Erika Vacchelli[a,b,c*]

[a]Equipe Labellisée Par La Ligue Contre Le Cancer, Université De Paris, Sorbonne Université, INSERM U1138, Centre De Recherche Des Cordeliers, Paris, France; [b]Metabolomics and Cell Biology Platforms, Gustave Roussy Cancer Campus, Villejuif, France; [c]Gustave Roussy Cancer Campus, Villejuif, France; [d]Faculty of Medicine Kremlin Bicêtre, Université Paris Sud, Paris Saclay, Kremlin Bicêtre, France; [e]Department of Radiation Oncology, Weill Cornell Medical College, New York, NY, USA; [f]Sandra and Edward Meyer Cancer Center, New York, NY, USA; [g]Caryl and Israel Englander Institute for Precision Medicine, New York, NY, USA; [h]Department of Dermatology, Yale School of Medicine, New Haven, CT, USA; [i]Université De Paris, Paris, France; [j]Equipe Labellisée Ligue Contre Le Cancer, INSERM, Villejuif, France; [k]Center of Clinical Investigations in Biotherapies of Cancer (CICBT) 1428, Villejuif, France; [l]AP-HP, Hôpital Européen Georges Pompidou, Paris, France; [m]Suzhou Institute for Systems Medicine, Chinese Academy of Medical Sciences, Suzhou, China; [n]Karolinska Institute, Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden

ABSTRACT

Toll-like receptor 3 (TLR3) is a pattern recognition receptor that senses exogenous (viral) as well as endogenous (mammalian) double-stranded RNA in endosomes. On activation, TLR3 initiates a signal transduction pathway that culminates with the secretion of pro-inflammatory cytokines including type I interferon (IFN). The latter is essential not only for innate immune responses to infection but also for the initiation of antigen-specific immunity against viruses and malignant cells. These aspects of TLR3 biology have supported the development of various agonists for use as stand-alone agents or combined with other therapeutic modalities in cancer patients. Here, we review recent preclinical and clinical advances in the development of TLR3 agonists for oncological disorders.

Abbreviations: cDC, conventional dendritic cell; CMT, cytokine modulating treatment; CRC, colorectal carcinoma; CTL, cytotoxic T lymphocyte; DC, dendritic cell; dsRNA, double-stranded RNA; FLT3LG, fms-related receptor tyrosine kinase 3 ligand; HNSCC, head and neck squamous cell carcinoma; IFN, interferon; IL, interleukin; ISV, *in situ* vaccine; MUC1, mucin 1, cell surface associated; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; polyA:U, polyadenylic:polyuridylic acid; polyI:C, polyriboinosinic: polyribocytidylic acid; TLR, Toll-like receptor

ARTICLE HISTORY
Received 3 May 2020
Accepted 13 May 2020

KEYWORDS
Dendritic cells; immune checkpoint blockers; polyA:U; polyI:C; Riboxxol

Introduction

Toll-like receptors (TLRs) are an evolutionarily conserved family of pattern recognition receptors (PRRs)[1–4] that detect conserved molecular motifs in microbial and endogenous products, which are generally referred to as microbe- or damage-associated molecular patterns (MAMPs or DAMPs), respectively.[5–11]

Since the initial discovery of Toll as a *Drosophila melanogaster* receptor with antifungal activity,[12–14] no less than 13TLRs have been characterized in mammalian organisms, 10 of which are also encoded by the human genome.[5,15] Mammalian TLRs localize either to the cell surface (TLR1, TLR2, TLR4, TLR6, TLR10) or within endosomal compartments (TLR3, TLR7, TLR8, TLR9).[5,16] Such endosomal TLRs are specialized in the recognition of potentially pathogenic nucleic acids, based on three general principles (1) availability (a function of initial concentration and degradation by endogenous nucleases), (2) localization (of both nucleic acids and TLRs) and (3) structural features (secondary nucleic acid conformations as well as chemical modifications).[17–19] On activation, nucleic acid-sensing TLRs initiate a signal transduction cascade that culminates with the secretion of numerous pro-inflammatory cytokines including type I interferon (IFN), which promote both innate and adaptive immune responses.[20–26]

Double-stranded RNA (dsRNA) molecules are the prototypic ligands of TLR3,[27] and activation occurs upon dsRNA binding to TLR3 leucine-rich repeats (LLR) domain.[28,29] Several mechanisms have been suggested to account for the accumulation of TLR3-activitory dsRNA molecules within endosomes, including clathrin-dependent endocytosis,[30,31] uptake of apoptotic bodies from infected cells,[32,33] autophagic uptake of dsRNA from the cytosol and trafficking to endosomes in the context of inhibited lysosomal degradation,[34–36] and formation of dsRNA complexes with cathelicidin antimicrobial peptide (CAMP).[37,38]

Upon ligand binding, the cytoplasmic Toll/IL-1 receptor (TIR) domain of TLR3[39,40] engages toll-like receptor adaptor molecule 1 (TIRAM1, best known as TRIF) and toll-like receptor adaptor molecule 2 (TIRAM2, best known as TRAM) to initiate a signal transduction cascade that culminates with the activation of TANK binding kinase 1 (TBK1)[41] and consequent derepression of interferon regulatory factor 3 (IRF3),[42] IRF7[43] and nuclear factor-kappa

CONTACT Guido Kroemer kroemer@orange.fr Equipe Labellisée Par La Ligue Contre Le Cancer, Université De Paris, Sorbonne Université, INSERM U1138, Centre De Recherche Des Cordeliers, Paris, France

*Share senior co-authorship

B (NF-κB).[44–46] Moreover, active TLR3 can signal via the mitogen-activated protein kinase (MAPK) system[5,47,48] to initiate transcriptional programs downstream of Jun proto-oncogene, AP-1 transcription factor subunit (JUN, best known as AP-1)[49] and cAMP responsive element binding protein 1 (CREB1).[50,51] Thus, TLR3 signaling favors the synthesis and secretion of a panoply of pro-inflammatory cytokines including not only type I IFN but also tumor necrosis factor (TNF), interleukin 6 (IL-6) and various chemokines such as C-C motif chemokine ligand 2 (CCL2) and C-X-C motif chemokine ligand 1 (CXCL1).[52–57] Of note, unlike other TLRs, TLR3 signaling appears to require tyrosine phosphorylation upon dsRNA recognition,[58] and operates exclusively via an MYD88 innate immune signal transduction adaptor (MYD88)-independent mechanism.[59,60]

Defective TLR3 activity contributes to numerous pathologies, including chronic inflammation, sepsis, autoimmune disorders and cancer.[61–66] Specifically, loss-of-function TLR3 polymorphisms have been associated with an increased risk for breast carcinoma,[67] cervical cancer,[68] oral squamous cell carcinoma,[69] hepatocellular carcinoma (HCC),[70] and colorectal carcinoma (CRC);[71] as well as with poor disease outcome in patients with CRC[72] and non-small cell lung carcinoma (NSCLC).[73] Moreover, high expression levels of TLR3 or TRIF have been shown to convey positive prognostic value in patients with HCC,[74,75] and neuroblastoma,[76] while TLR3 expression has attributed predictive value in a cohort of women with breast carcinoma treated with adjuvant radiotherapy plus a TLR3 agonist.[77,78] Finally, several studies have demonstrated that the emission of DAMPs by dying cancer cells, either spontaneously or following treatment, enables the initiation of an efficient and durable anticancer immune response through the activation of TLRs and other PRRs on immune cells of the host.[79–82] Thus, TLR3 stimulation stands out as a promising strategy to (re)instance cancer immunosurveillance[83] and demonstrated potential especially as an adjuvant to therapeutic tumor-targeting vaccines.[84–91] However, whereas TLRs located at the plasma membrane can be actioned with small molecules and antibodies, targeting nucleic-acid sensing TLRs, such as TLR3, require modified oligonucleotides.[87] Indeed, besides natural dsRNA molecules, TLR3 also recognizes synthetic dsRNA analogs,[48] such as polyriboinosinic:polyribocytidylic acid (polyI:C),[92] polyadenylic:polyuridylic acid (polyA:U),[93] polyriboinosinic-polyribocytidylic acid-polylysine carboxymethylcellulose (polyI:CLC, best known as Hiltonol™)[94] and polyI:C_{12}U (best known as Ampligen™ or rintatolimod),[95,96] all of which have been consistently used to induce TLR3 signaling *in vitro* and *in vivo*.[97–101]

Over the past few years, numerous studies have confirmed the ability of TLR3 agonists to support the activation of tumor-specific immune responses in mice and patients, especially when combined with other therapeutic modalities.[90,102-104] However, the clinical efficacy of this approach remains limited, potentially reflecting the existence of numerous, non-overlapping immunosuppressive pathways that must be simultaneously disabled to allow for therapeutically relevant tumor-targeting immune responses in patients.[83,105,105-107,109] Here, we discuss recent progress on the development of TLR3 agonists for cancer therapy.

Preclinical advances

In this section, we summarize the key preclinical studies on the ability of TLR3 agonists to (re)instate anticancer immunosurveillance, which have been released since the publication of the latest Trial Watch dealing with this topic.[90]

PolyI:C and polyA:U, dsRNA mimetics

PolyI:C was originally synthesized in the mid-1960s by Hilleman and colleagues.[108] This synthetic dsRNA consists of an RNA duplex composed of one inosinic acid polymer and one cytidylic acid polymer. The treatment of immature dendritic cells (DCs) with TLR3 induces their functional maturation, as demonstrated by a reduction in phagocytic/pinocytic capacity coupled to increased expression of co-stimulatory molecules (*e.g.*, CD80 and CD86), maturation markers (*e.g.*, CD83) and immunostimulatory cytokines (*e.g.*, IL-12).[109] Interestingly, TLR3 is highly expressed both by a subset of mouse ($CD8\alpha^+$)[110,112] and human ($CD141^+$)[113,114] DCs commonly known as type I conventional DCs (cDC1s).[86,115-117] This basic leucine zipper ATF-like transcription factor 3 (BATF3)-dependent DC lineage has been extensively studied for its ability to efficiently cross-prime $CD8^+$ cytotoxic T lymphocytes (CTLs).[118–120] In line with these observations, Kline et al. have recently demonstrated that intraperitoneal injection of polyI:C elicits robust anti-leukemia T cell immunity and considerably prolongs survival of leukemia-bearing mice upon the engagement of $CD8\alpha^+$ cDC1s.[121]

Several combinatorial regimens have been developed to increase the antineoplastic effects of polyI:C, some of which demonstrated pronounced therapeutic activity in preclinical models of melanoma[122,123] as well as CRC,[124,125] mammary,[124] and squamous carcinoma.[122] In particular, systemic administration of the DC growth factor fms-related receptor tyrosine kinase 3 ligand (FLT3 LG) followed by intratumoral polyI:C injections improved magnitude and duration of response to B-Raf proto-oncogene, serine/threonine kinase (BRAF) and CD274 (best known as PD-L1) blockade in mouse B16 melanomas, via a mechanism involving cDC1s.[123,126] Di and colleagues have recently evaluated the efficacy of polyI:C administered in combination with epithelial growth factor receptor (EGFR)vIII-targeted CAR-T cells,[127] both *in vitro* and in immunocompetent mice bearing subcutaneous CRC or orthotopic mammary cancer xenografts. In this setting, polyI:C significantly increased the levels of effector cytokines such as IL-2 and IFNγ, as well as the lytic activity of CAR-T cells while reducing the number and function of myeloid-derived suppressor cells (MDSC) in the peripheral blood and spleen.[124] Interestingly, Guinn and colleagues have recently reported that IFNγ synergizes with polyI:C in limiting the growth of mouse B16 melanoma and human UM-SCC1 squamous carcinoma cells *in vitro*, suggesting yet another mechanism through which polyI:C may mediate antineoplastic effects *in vivo*.[122] Along similar lines, polyI:C and the microtubular poison paclitaxel[128] have been reported to synergistically inhibit the growth of paclitaxel-resistant human CRC cells *in vitro* through a pathway that involves enhanced interferon beta 1 (IFNB1) expression downstream of TLR3.[125] These latter

findings suggest that the ability of polyI:C (and potentially other TLR3 agonists) to activate innate immune pathways in malignant cells may contribute to its therapeutic efficacy,[129] which is generally attributed to the engagement of the host immune system. Further supporting this possibility, TLR3 is known to promote apoptosis[98,130,131] as well as a non-apoptotic form of cancer cell death known as necroptosis,[132–134] which (at least in some settings) has therapeutic value.[135,136]

Several laboratories have recently focused their attention on the design of innovative delivery platforms for polyI:C. Thus, Aznar and collaborators have developed a nanoplexed formulation of polyI:C complexed with polyethylenimine (BO-112), which induces the apoptotic demise of cancer cells accompanied by features of immunogenic cell death (ICD).[137] Intratumoral injection of BO-112 to mouse MC38 CRCs, 4T1 mammary carcinomas and B16 melanomas promoted tumor infiltration by $CD8^+$ CTLs and established at least some degree of disease control dependent on IFNγ,[137] fostering clinical testing in patients with solid tumors (NCT02828098). Alongside, polyI:C has been delivered together with cancer cell lysates[138] with an injectable and self-assembled poly (*L*-valine) hydrogel. This vaccine formulation allowed for the recruitment, activation and maturation of DCs *in vivo* as it improved antigen persistence at the injection site and antigen drainage to lymph nodes.[139] Thus, subcutaneous administration of the hydrogel-based vaccine to melanoma-bearing mice mediated robust antineoplastic effects through a proficient CTL response.[139]

Similar to polyI:C, polyA:U was synthesized by the Hilleman's laboratory in the mid-1960s.[110] This double-stranded polyribonucleotide, composed of equimolar polyadenylic acid and polyuridylic acid, was extensively investigated in the 1980s[140-142] as the first clinical trials investigating the safety and preliminary activity of polyI:C documented side effects including fever, nausea and hypotension on systemic administration.[142,143] Even though polyI:C is more potent than polyA:U,[93,99,144] the latter is still used in several studies, at least in part because of its reduced toxicity. Supporting the ability of polyA:U to enhance anticancer immune responses, adjuvant polyA:U administration has been associated with a significant reduction in the risk for metastatic relapse amongst breast cancer patients with TLR3-expressing tumors.[77] Recently, Roselli et *al.* have reported that the intratumoral administration of naked polyA:U delays the growth of B16 melanomas *in vivo*, and significantly prolongs the survival of tumor-bearing mice.[112] This effect appears to be orchestrated by multiple changes within the lymphoid compartment of the tumor microenvironment, encompassing an increased abundance of $CD8^+$ CTLs expressing the effector molecule granzyme B (GZMB),[145] a reduction in the relative amount of tumor-infiltrating $CD4^+CD25^+FOXP3^+$ regulatory T (T_{REG}) cells[105] (with respect to $CD8^+$ cells), an improved proliferation of tumor-antigen specific $CD8^+$ CTLs, as well as an enhanced expression of programmed cell death 1 (PDCD1, best known as PD-1)[146] and its main ligand CD274. These findings suggest that, similar to numerous chemotherapeutic agents that promote PD-L1 expression,[147,148] polyA:U might be advantageously combined with immunotherapies blocking the PD-1/PD-L1 axis.[112,149,150]

Ampligen™, an analogue of polyI:C

Ampligen™ (also known polyI:C_{12} U, AMP-516 or rintatolimod) was synthesized in the 1970 s by William A. Carter by adding unpaired uracil and guanine bases to the classical polyI:C structure.[151] Ampligen™, which drives type I IFN production and IFNB1-dependent type I helper (T_H1) responses with reduced toxicity as compared to polyI:C,[151] was originally intended for the treatment of chronic fatigue syndrome (CFS), a complex disorder characterized by extreme fatigue,[152,153] and only later it was used as a TLR3 agonist for other indications including cancer.[151] Recently, Tomasicchio *et al.* haver recruited 12 women with stage 1 to 3 breast carcinoma for *ex vivo* studies on their DC compartment, ultimately demonstrating that optimal DC maturation required a combinatorial treatment including Ampligen™, an autologous tumor cell lysate, the TLR7/8 agonist resiquimod,[154,155] and a cytokine cocktail encompassing IFNγ, IFNα, IL-1β and CD40 ligand (CD40 L). DCs matured *ex vivo* under these conditions produced high levels of IL-12 and could enhance antigen-specific $CD8^+$ CTL responses against erb-B2 receptor tyrosine kinase (ERBB2, better known as HER-2)[156] and mucin 1, cell surface associated (MUC1)[157] leading to the destruction of autologous breast cancer cells *in vitro*.[158]

Riboxxol, a dsRNA duplex

Riboxxol (also known as RGIC®50) is a synthetic dsRNA containing cytosines, inosines and guanosines that potently stimulates the secretion of several pro-inflammatory cytokines and improves the ability of mouse and human cDC1s to stimulate T cell proliferation.[159] Schau and colleagues have recently designed a targeted delivery system consisting of neutravidin (a deglycosylated version of avidin)[160,161] conjugated to monobiotinylated Riboxxol and a humanized anti-prostate stem cell antigen (PSCA) single-chain antibody derivative. These nanoparticle-like immunoconjugates, which were called "rapid inducer of cellular inflammation and apoptosis" (RICIA) were able to specifically deliver Riboxxol to PSCA-expressing tumor cells and induce a type I IFN response coupled to apoptotic cell death.[162]

ARNAX, a double-stranded RNA mimic

ARNAX is a TLR3 agonist originally developed by Matsumoto and collaborators that consists of a phosphorothioate oligodeoxynucleotide (ODN)-guided dsRNA.[163] Unlike polyI:C, this chimeric molecule does not activate intracellular RNA sensors other than TLR3[164,165] such as DExD/H-box helicase 58 (DDX58 best known as RIG-I) and interferon induced with helicase C domain 1 (IFIH1, best known as MDA5) and hence presents reduced toxicity *in vitro* and *in vivo*.[166–168] Takeda and collaborators have recently demonstrated that ARNAX synergizes with a model peptide vaccine and a CD274 (best known as PD-L1) blockers in the eradication of various mouse tumors established in immunocompetent hosts.[169]

Translational and clinical progress

Results from a number of translational and clinical studies addressing the safety and therapeutic potential of TLR3

agonists have been reported in the peer-reviewed literature since the publication of the latest Trial Watch on this topic (October 2018).[90] Here, we discuss some of these studies with a focus on findings and concepts that recapitulate the current state-of-the-art.

Translational studies

Recent immunohistochemical studies demonstrate that high TLR3 expression by tumor cells correlates with favorable disease outcome in a cohort of 194 patients with early-stage NSCLC, whereas TLR3 expression on immune cells, infiltrating the tumor bed, is associated with poor overall survival.[170] At least in part, these observations appear to reflect the ability of TLR3 activation to drive apoptotic cell death in cancer cells, as demonstrated *in vitro* as well as by the immunohistochemical quantification of active caspase 3 (CASP3), a key mediator of apoptosis,[171–173] in tumor biopsies. Tan and colleagues have recently reported that nasopharyngeal carcinoma (NPC) biopsies exhibit increased *TLR3* mRNA levels as compared to healthy nasopharyngeal tissues,[54] which appears to constitute an actionable therapeutic target. Indeed, Hiltonol™ synergized with the endothelial growth factor receptor (EGFR)-specific antibody cetuximab in (1) maturation of DCs, (2) activation of natural killer (NK) cell-dependent antibody-dependent cellular cytotoxicity (ADCC) and cytotoxicity, and tumor infiltration by EGFR-specific CTLs.[54] None of these effects was affected by TLR3 polymorphisms (e.g., L412F or C829T), pleading in favor of a broad use of Hiltonol™ against NPC.[54] Finally, Hammerich et al. developed an *in situ* vaccine (ISV) approach, combining recombinant human FLT3LG, local radiotherapy, and Hiltonol™ that robustly activated an anticancer immune response amenable to boosting with PD-1 blockers in lymphoma bearing mice.[174] These results prompted the initiation of a hitherto ongoing clinical trial (NCT01976585) enrolling patients with advanced stage indolent non-Hodgkin's lymphoma (iNHL).

Clinical studies

Preliminary results for the aforementioned NCT01976585 clinical trial (testing a Hiltonol™-based ISV approach in patients with iNHL) suggested that both responders and non-responders to the ISV develop (at least some degree of) anticancer immunity, based on analysis of peripheral blood mononuclear cells (PBMCs) for maturation and exhaustion markers in DCs and CTLs.[174] However, it seems that a population of $PD\text{-}1^{+}CD8^{+}$ T lymphocytes emerges in non-responders, potentially explaining why of 11 patients included in this preliminary analysis, no less than 8 experienced partial or complete lymphoma regression in the presence of a PD-1 blocker.[174] Conversely, only six patients showed stable disease or minor regression (lasting 3 to 18 months) at distant untreated tumors whereas two patients progressed. Altogether, these findings suggest that iNHL patients might benefit from a Hiltonol™-based ISV approach combined with PD-1 blockers.

Weed et al. reported preliminary results from a Phase I clinical assay testing the safety and immunological efficacy of a MUC1-targeting peptide vaccine admixed with Hiltonol™ and combined with a phosphodiesterase type 5 (PDE5) inhibitor (tadalafil)[175] in subjects with head and neck squamous cell carcinoma (HNSCC) (NCT02544880).[176] While no severe side effects and treatment-limiting toxicities were documented, this regimen increased the amount of activated tumor-infiltrating lymphocytes (TIL) and reduced the levels of $PD\text{-}L1^{+}$ macrophages at the tumor edge,[176] suggesting that the addition of a PD-1- or PD-L1-taregting immune checkpoint blockers may be useful also in this setting. Alongside, a pilot study on patients with metastatic HNSCC and melanoma who received intratumoral or intramuscular Hiltonol™ reported clinical benefits for at least one of the 8 individuals enrolled in this trial, coupled to moderate side effects (such as inflammation at the injection site and fatigue) as well as increased levels of CD4, CD8, PD-1, and PD-L1 in tumors, confirming the activation of systemic immunity.[177]

Keskin and colleagues reported the results of a Phase Ib clinical trial in which newly diagnosed glioblastoma patients with unmethylated methylguanine methyltransferase (*MGMT*) received a personalized neoantigen vaccine, previously administered to melanoma patients,[178–180] admixed with Hiltonol™.[181] This regimen generated strong intratumoral T-cell responses even though glioblastoma is generally viewed as an immunological 'desert',[182] suggesting that robustly adjuvanted neoepitope-targeting vaccines may constitute a valid approach for the treatment of glioblastoma, especially in combination with immune checkpoint blockers.[183] Apparently at odds with this notion, Boydell and colleagues reported that a multipeptide vaccine (IMA950) admixed with Hiltonol™, administered prior to the vascular endothelial growth factor A (VEGFA)-targeting antibody bevacizumab,[184,185] failed to improve the therapeutic activity of the latter in high-grade glioma patients, as assessed by progression-free and overall survival (NCT01920191).[186]

Melssen *et al.* investigated the safety, immunogenicity and preliminary efficacy of a multipeptide vaccine[187,188] admixed with (1) Hiltonol™ and/or incomplete Freund's adjuvant (IFA), or (2) the mixed TLR2/TLR4 agonist lipopolysaccharide (LPS)[189,190] and/or IFA in melanoma patients (NCT01585350).[191] Preliminary findings from this study indicate that Hiltonol™ plus IFA can induce durable peptide-specific $CD8^{+}$ T cell responses in the absence of considerable side effects (dose-limiting toxicities were documented in only 11% of the subjects). Finally, one Phase I study evaluated the therapeutic effect of TLR3 agonists in pediatric cancers (NCT01188096).[192] Of note, all six patients affected by type I neurofibromatosis (among the 23 enrolled in the trial) tolerated Hiltonol™ as a stand-alone intervention (mild side effects included fever, pain at site of injection, erythema and myalgias),[192] supporting the planification of a Phase II study for this specific oncological indication.

Overall, this translational and clinical literature supports the notion that TLR3 agonists may favor the ability of therapeutic vaccines to (re)activate immunosurveillance in (at least some) patients affected by solid tumors, although efficacy in the absence of immune checkpoint blockers remains limited.

Recently initiated clinical trials

Since the submission of the latest Trial Watch dealing with this topic (October 2018),[90] only 8 clinical studies encompassing

the administration of TLR3 agonists to cancer patients have been initiated (source http://clinicaltrials.gov/), all of which involved either Hiltonol™ (4 studies),[193–195] or Ampligen™ (4 studies)[151] (Table 1).

In particular, NCT04119830 aims at evaluating the toxicity of Ampligen™ in combination with the PD-1 blocker pembrolizumab,[196,197] as well as the impact of this regimen on progression-free and overall survival in patients affected by metastatic, refractory or unresectable CRC.[198] Patients with CRC-derived liver metastases[199] are also being enrolled in NCT03403634, Phase II study involving the administration of a recombinant IFNα-2b (rIFNα-2b)- and Ampligen™-based cytokine modulating treatment (CMT)[200] plus the nonsteroidal anti-inflammatory drug celecoxib.[201] In this study, the impact of treatment on the immune microenvironment is evaluated by the immunohistochemical assessment of CTL/T_{REG} cell ratio.[202–204]

In an analogous manner, HLA-$A2^+$ individuals with primary PD-1-resistant or refractory melanoma are being enrolled in NCT04093323, a Phase II study combining the aforementioned CMT (rIFNα-2b, Ampligen™, and celecoxib) with a vaccine in which cDC1s are loaded with tumor blood vessel-derived antigenic peptides. Twelve weeks after treatment initiation, patients with progressive disease may receive the cytotoxic T-lymphocyte associated protein 4 (CTLA4) blocker ipilimumab[205-209] with or without a PD-1/PD-L1 inhibitor. Patients experiencing complete responses or stable disease may receive PD-1/PD-L1 inhibitors or an appropriate alternative care. A modified variant of the rIFNα-2b-based CMT that involves aspirin[210,212] instead of celecoxib is also being tested in prostate cancer patients scheduled for radical prostatectomy (NCT03899987). The objectives of this window-of-opportunity study aim at assessing safety, antitumor activity, and immunomodulatory effects.

Hiltonol™ is being tested as a stand-alone intervention only in a Phase I clinical assay involving the intravenous administration of the drug to patients affected by malignant pleural mesothelioma prior to surgical resection (NCT04345705). All the other clinical trials recently initiated to test Hiltonol™ in patients with cancer co-involve indeed either a PD-1 blocker[197,213] (NCT03789097 and NCT03835533) or an agonist for the co-stimulatory T cell receptor CD27 (varlilumab, also known as CDX-1127)[214,215] (NCT03617328).

In particular, Hiltonol™ is being administered in combination with radiotherapy and rhFLT3LG as *in situ* vaccine supported by systemic pembrolizumab[216] to patients affected by metastatic breast cancer, HNSCC and NHL in the context of a Phase I/II assay (NCT03789097). This combinatorial regimen includes three therapies directed against a "target site": (1) rhFLT3LG, known also by the name of CDX-301,[217,218] that specifically recruits and expands DCs, (2) radiation therapy to the tumor and the draining lymph node (administered at a 10–20 times lower dose compared to the standard for patients with this specific type of neoplasm),[78] and (3) Hiltonol™, which should activate the immune cells recruited into the tumor by rhFLT3LG and radiation. Of note, pembrolizumab is already approved by the U.S. Food and Drug Administration (FDA) for the treatment of several neoplasms including HNSCC, but is not effective against metastatic breast carcinomas and NHL.[219,220]

Along similar lines, Hiltonol™ is currently being tested in individuals with metastatic castration-resistant prostate cancer[221-224] simultaneously receiving the PD-1 blocker nivolumab,[225] rhFLT3LG and stereotactic body radiation therapy (SBRT)[226] (NCT03835533). The principal purpose of this study is to monitor the safety and efficacy of different immunotherapy-based combinatorial regimens: one arm (cohort B) receives the aforementioned Hiltonol™-based regimen; a second arm (cohort A) receives nivolumab together with NKTR-214, an IL-2 agonist targeting interleukin 2 receptor subunit beta (IL2RB, also known as CD122)[227–229]; and (3) a third arm (cohort C) receives nivolumab together with rhFLT3LG and INO-5151, a combined formulation of INO-5150 – a DNA vector expressing kallikrein-related peptidase 3 (KLK3, best known as PSA)[230] and folate hydrolase 1 (FOLH1, best known as PSMA)[231] – and INO-9012 – a DNA vector expressing IL-12.[232]

Finally, the Phase I/II clinical study NCT03617328 evaluates the safety, efficacy and immunogenicity of a peptide vaccine comprised six class II MHC-restricted peptides (6MHP) in patients with melanoma. In this trial, vaccination is adjuvanted with Hiltonol™ and montanide ISA-51,[233] as well as with varlilumab.[234–236]

Table 1. Clinical trials currently testing TLR3 agonists in oncological indications.

Agonist	Indication(s)	Phase(s)	Route	Recruitment	Interventions	Ref
Ampligen	Colorectal cancer	II	*i.v.*	Not yet recruiting	Combined with pembrolizumab	NCT04119830
				Recruiting	Combined with celecoxib and rIFNα-2b	NCT03403634
	Melanoma	II	*i.v.*	Not yet recruiting	Combined with DC vaccination, celecoxib, PD-1/PD-L1 inhibitors and rIFNα-2b	NCT04093323
	Prostate cancer	II	*i.v.*	Recruiting	Combined with aspirin ± rIFNα-2b	NCT03899987
Hiltonol	Breast cancer HNSCC NHL	I/II	*i.t.*	Recruiting	Combined with pembrolizumab, radiotherapy and rhFLT3LG	NCT03789097
	Melanoma	I/II	*i.d. s.c.*	Recruiting	Combined with multipeptide vaccine, montanide ISA-51 ± varlilumab	NCT03617328
	Mesothelioma	I	*i.t.*	Not yet recruiting	As single agent	NCT04345705
	Prostate cancer	I	*i.m.*	Recruiting	Combined with nivolumab, rhFLT3LG and SBRT	NCT03835533

Abbreviations: HNSCC, head and neck squamous cell carcinoma; *i.d.*, intra derma; *i.m.*, intra musculum; *i.t.*, intra tumorem; *i.v.*, intra venam; NHL, non-Hodgkin's lymphoma; rhFLT3LG, recombinant human fms-like tyrosine kinase 3 ligand; rIFNα-2b, recombinant interferon α-2b; SBRT, stereotactic body radiation therapy; *s.c.*, sub cutem.

The status of the following clinical trials discussed in our previous Trial Watches dealing with TLR3 agonists[90] has changed during the past 19 months: NCT02334735, NCT02544880, NCT02721043, NCT02826434, NCT02873819, NCT02897765, NCT03162562, NCT03358719, NCT03380871 and NCT03597282 which are now listed as "Active, not recruiting"; NCT02886065, which is listed as "Recruiting"; NCT02134925 which is currently listed as "Active, not recruiting with results"; NCT02149225, which is listed as "Completed"; NCT03206047 and NCT03300817, which have been "Suspended"; NCT02061449 which has been "Terminated"; as well as NCT02754362, which has been "Withdrawn" (source http://clinicaltrials.gov/).

NCT02134925 is a randomized Phase II study evaluating a MUC1-targeting peptide vaccine admixed with Hiltonol™ *versus* placebo in patients with newly diagnosed advanced colon polyps. Preliminary results from 110 patients enrolled in the study suggest that vaccination induces superior levels of circulating MUC1-specific IgG, and some degree of reduction in adenoma recurrence rate (56.3% versus 66.0%) (source http://clinicaltrials.gov/). To the best of our knowledge, the results of NCT02149225 (a Phase I study investigating the safety and preliminary efficacy of a Hiltonol™-adjuvanted vaccine in glioblastoma patients) have not been disseminated yet. NCT03206047 (a Phase I/II trial testing Hiltonol™-adjuvanted DC-targeting vaccine in women with recurrent ovarian, fallopian tube, or primary peritoneal cancer) and NCT03300817 (a Phase I study testing a Hiltonol™-adjuvanted, MUC1-targeting vaccine to prevent lung cancer in former and current smokers) have been suspended for undisclosed reasons or to ensure patient safety during the Covid19 epidemics,[237–239] respectively (source http://clinicaltrials.gov/). NCT02061449 (a Phase I study investigating Hiltonol™ plus radiation in patients with advanced cutaneous T cell lymphoma) has been terminated because of poor accrual. Finally, NCT02754362 (a Phase II trial testing Hiltonol™ and montanide ISA-51 in support of a multipeptide vaccine administered prior to bevacizumab in glioblastoma patients) has been withdrawn due to personnel changes (source http://clinicaltrials.gov/).

Concluding remarks

The blockade of co-inhibitory T cell receptors or their ligands, as achieved with immune checkpoint inhibitors targeting CTLA4, PD-1 and PD-L1, has been a major success in the treatment of patients with various tumors. However, at this stage, immunotherapies only provide long-term clinical benefits to a minority of patients, calling for a drastic amelioration of standard of care. In this context, numerous studies have been launched to identify additional immunosuppressive or immunostimulatory circuitries that can be drugged. As discussed in the present Trial Watch, TLR3 stands out as a promising target for the (re)elicitation of anticancer immunosurveillance. However, the existence of numerous immunosuppressive circuitries that enable tumor progression and resistance to conventional therapies considerably limits the efficacy of TLR3 agonists employed as stand-alone agents, as well as of vaccines adjuvanted with TLR3 agonists, to mediate clinically relevant effects. We surmise that the development of properly scheduled combinatorial regimens involving multiple immunotherapeutic agents (notably, immune checkpoint blockers and agents that recruit and expand DCs) will be required for harnessing the full antineoplastic potential of TLR3 agonists.

Acknowledgments

LG is supported by a Breakthrough Level 2 grant from the US Department of Defense (DoD), Breast Cancer Research Program (BRCP) (#BC180476P1), by the 2019 Laura Ziskin Prize in Translational Research (#ZP-6177, PI: Formenti) from the Stand Up to Cancer (SU2C), by a Mantle Cell Lymphoma Research Initiative (MCL-RI, PI: Chen-Kiang) grant from the Leukemia and Lymphoma Society (LLS), by a startup grant from the Dept. of Radiation Oncology at Weill Cornell Medicine (New York, US), by a Rapid Response Grant from the Functional Genomics Initiative (New York, US), by industrial collaborations with Lytix (Oslo, Norway) and Phosplatin (New York, US), and by donations from Phosplatin (New York, US), the Luke Heller TECPR2 Foundation (Boston, US) and Sotio a.s. (Prague, Czech Republic). GK is supported by the Ligue contre le Cancer (équipe labellisée); Agence National de la Recherche (ANR) – Projets blancs; ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; AMMICa US23/CNRS UMS3655; Association pour la recherche sur le cancer (ARC); Association "Le Cancer du Sein, Parlons-en!"; Cancéropôle Ile-de-France; Chancelerie des universités de Paris (Legs Poix), Fondation pour la Recherche Médicale (FRM); a donation by Elior; European Research Area Network on Cardiovascular Diseases (ERA-CVD, MINOTAUR); Gustave Roussy Odyssea, the European Union Horizon 2020 Project Oncobiome; Fondation Carrefour; High-end Foreign Expert Program in China (GDW20171100085), Institut National du Cancer (INCa); Inserm (HTE); Institut Universitaire de France; LeDucq Foundation; the LabEx Immuno-Oncology (ANR-18- IDEX-0001); the RHU Torino Lumière; the Seerave Foundation; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); and the SIRIC Cancer Research and Personalized Medicine (CARPEM).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

ORCID

Lorenzo Galluzzi http://orcid.org/0000-0003-2257-8500
Laurence Zitvogel http://orcid.org/0000-0003-1596-0998
Guido Kroemer http://orcid.org/0000-0002-9334-4405

References

1. Leulier F, Lemaitre B. Toll-like receptors–taking an evolutionary approach. Nat Rev Genet. 2008;9(3):165–178. doi:10.1038/nrg2303.
2. Roach JC, Glusman G, Rowen L, Kaur A, Purcell MK, Smith KD, Hood LE, Aderem A. The evolution of vertebrate Toll-like receptors. Proc Natl Acad Sci U S A. 2005;102(27):9577–9582. doi:10.1073/pnas.0502272102.
3. Brennan JJ, Gilmore TD. Evolutionary origins of toll-like receptor signaling. Mol Biol Evol. 2018;35(7):1576–1587. doi:10.1093/molbev/msy050.
4. Wang PH, He JG. Nucleic acid sensing in invertebrate antiviral immunity. Int Rev Cell Mol Biol. 2019;345:287–360.
5. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. Front Immunol. 2014;5:461. doi:10.3389/fimmu.2014.00461.
6. Kumar H, Kawai T, Akira S. Toll-like receptors and innate immunity. Biochem Biophys Res Commun. 2009;388(4):621–625. doi:10.1016/j.bbrc.2009.08.062.

7. Ramadan A, Land WG, Paczesny S. Editorial: danger Signals Triggering Immune Response and Inflammation. Front Immunol. 2017;8:979. doi:10.3389/fimmu.2017.00979.
8. Garg AD, Galluzzi L, Apetoh L, Baert T, Birge RB, Bravo-San Pedro JM, Breckpot K, Brough D, Chaurio R, Cirone M, et al. Molecular and translational classifications of DAMPs in immunogenic cell death. Front Immunol. 2015;6:588. doi:10.3389/fimmu.2015.00588.
9. Gong T, Liu L, Jiang W, Zhou R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. Nat Rev Immunol. 2020;20(2):95–112. doi:10.1038/s41577-019-0215-7.
10. Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P. Immunogenic cell death and DAMPs in cancer therapy. Nat Rev Cancer. 2012;12(12):860–875. doi:10.1038/nrc3380.
11. Krysko O, Love Aaes T, Bachert C, Vandenabeele P, Krysko DV. Many faces of DAMPs in cancer therapy. Cell Death Dis. 2013;4(5):e631. doi:10.1038/cddis.2013.156.
12. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell. 1996;86(6):973–983. doi:10.1016/S0092-8674(00)80172-5.
13. Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature. 1997;388(6640):394–397. doi:10.1038/41131.
14. Valanne S, Wang JH, Ramet M. The Drosophila toll signaling pathway. J Immunol. 2011;186(2):649–656. doi:10.4049/jimmunol.1002302.
15. Barreiro LB, Ben-Ali M, Quach H, Laval G, Patin E, Pickrell JK, Bouchier C, Tichit M, Neyrolles O, Gicquel B, et al. Evolutionary dynamics of human Toll-like receptors and their different contributions to host defense. PLoS Genet. 2009;5(7):e1000562. doi:10.1371/journal.pgen.1000562.
16. Minton K. Regulation of endosomal TLRs. Nat Rev Immunol. 2019;19(11):660–661. doi:10.1038/s41577-019-0229-1.
17. Chung H, Calis JJA, Wu X, Sun T, Yu Y, Sarbanes SL, Dao Thi VL, Shilvock AR, Hoffmann -H-H, Rosenberg BR, et al. Human ADAR1 prevents endogenous RNA from triggering translational shutdown. Cell. 2018;172(4):811–24 e14. doi:10.1016/j.cell.2017.12.038.
18. Schlee M, Hartmann G. Discriminating self from non-self in nucleic acid sensing. Nat Rev Immunol. 2016;16(9):566–580. doi:10.1038/nri.2016.78.
19. Vanpouille-Box C, Hoffmann JA, Galluzzi L. Pharmacological modulation of nucleic acid sensors - therapeutic potential and persisting obstacles. Nat Rev Drug Discov. 2019;18:845–867.
20. Matz KM, Guzman RM, Goodman AG. The role of nucleic acid sensing in controlling microbial and autoimmune disorders. Int Rev Cell Mol Biol. 2019;345:35–136.
21. Sprooten J, Agostinis P, Garg AD. Type I interferons and dendritic cells in cancer immunotherapy. Int Rev Cell Mol Biol. 2019;348:217–262.
22. Blasius AL, Beutler B. Intracellular toll-like receptors. Immunity. 2010;32(3):305–315. doi:10.1016/j.immuni.2010.03.012.
23. Beutler BA. TLRs and innate immunity. Blood. 2009;113(7):1399–1407. doi:10.1182/blood-2008-07-019307.
24. Vercammen E, Staal J, Beyaert R. Sensing of viral infection and activation of innate immunity by toll-like receptor 3. Clin Microbiol Rev. 2008;21(1):13–25. doi:10.1128/CMR.00022-07.
25. Crouse J, Kalinke U, Oxenius A. Regulation of antiviral T cell responses by type I interferons. Nat Rev Immunol. 2015;15(4):231–242. doi:10.1038/nri3806.
26. Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G. Type I interferons in anticancer immunity. Nat Rev Immunol. 2015;15(7):405–414. doi:10.1038/nri3845.
27. Yu M, Levine SJ. Toll-like receptor, RIG-I-like receptors and the NLRP3 inflammasome: key modulators of innate immune responses to double-stranded RNA viruses. Cytokine Growth Factor Rev. 2011;22(2):63–72. doi:10.1016/j.cytogfr.2011.02.001.
28. Gay NJ, Gangloff M, Weber AN. Toll-like receptors as molecular switches. Nat Rev Immunol. 2006;6(9):693–698. doi:10.1038/nri1916.
29. Iwakiri D, Zhou L, Samanta M, Matsumoto M, Ebihara T, Seya T, Imai S, Fujieda M, Kawa K, Takada K, et al. Epstein-Barr virus (EBV)-encoded small RNA is released from EBV-infected cells and activates signaling from Toll-like receptor 3. J Exp Med. 2009;206(10):2091–2099. doi:10.1084/jem.20081761.
30. Itoh K, Watanabe A, Funami K, Seya T, Matsumoto M. The clathrin-mediated endocytic pathway participates in dsRNA-induced IFN-beta production. J Immunol. 2008;181(8):5522–5529. doi:10.4049/jimmunol.181.8.5522.
31. Watanabe A, Tatematsu M, Saeki K, Shibata S, Shime H, Yoshimura A, Obuse C, Seya T, Matsumoto M. Raftlin is involved in the nucleocapture complex to induce poly(I:C)-mediated TLR3 activation. J Biol Chem. 2011;286(12):10702–10711. doi:10.1074/jbc.M110.185793.
32. Salio M, Cerundolo V. Viral immunity: cross-priming with the help of TLR3. Curr Biol. 2005;15:R336–9.
33. Wang W, Wang WH, Azadzoi KM, Su N, Dai P, Sun J, Wang Q, Liang P, Zhang W, Lei X, et al. Activation of innate antiviral immune response via double-stranded RNA-dependent RLR receptor-mediated necroptosis. Sci Rep. 2016;6(1):22550. doi:10.1038/srep22550.
34. Galluzzi L, Green DR. Autophagy-independent functions of the autophagy machinery. Cell. 2019;177(7):1682–1699. doi:10.1016/j.cell.2019.05.026.
35. Hase K, Contu VR, Kabuta C, Sakai R, Takahashi M, Kataoka N, Hakuno F, Takahashi S-I, Fujiwara Y, Wada K, et al. Cytosolic domain of SIDT2 carries an arginine-rich motif that binds to RNA/DNA and is important for the direct transport of nucleic acids into lysosomes. Autophagy. pp.1–15. 2020. doi:10.1080/15548627.2020.1712109
36. Soreng K, Neufeld TP, Simonsen A. Membrane trafficking in autophagy. Int Rev Cell Mol Biol. 2018;336:1–92.
37. Singh D, Qi R, Jordan JL, San Mateo L, Kao CC. The human antimicrobial peptide LL-37, but not the mouse ortholog, mCRAMP, can stimulate signaling by poly(I:C) through a FPRL1-dependent pathway. J Biol Chem. 2013;288(12):8258–8268. doi:10.1074/jbc.M112.440883.
38. Takahashi T, Kulkarni NN, Lee EY, Zhang LJ, Wong GCL, Gallo RL. Cathelicidin promotes inflammation by enabling binding of self-RNA to cell surface scavenger receptors. Sci Rep. 2018;8(1):4032. doi:10.1038/s41598-018-22409-3.
39. Kawai T, Akira S. TLR signaling. Cell Death Differ. 2006;13(5):816–825. doi:10.1038/sj.cdd.4401850.
40. O'Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol. 2007;7(5):353–364. doi:10.1038/nri2079.
41. Bakshi S, Taylor J, Strickson S, McCartney T, Cohen P. Identification of TBK1 complexes required for the phosphorylation of IRF3 and the production of interferon beta. Biochem J. 2017;474(7):1163–1174. doi:10.1042/BCJ20160992.
42. Doyle S, Vaidya S, O'Connell R, Dadgostar H, Dempsey P, Wu T, Rao G, Sun R, Haberland ME, Modlin RL, et al. IRF3 mediates a TLR3/TLR4-specific antiviral gene program. Immunity. 2002;17(3):251–263. doi:10.1016/S1074-7613(02)00390-4.
43. Ning S, Pagano JS, Barber GN. IRF7: activation, regulation, modification and function. Genes Immun. 2011;12(6):399–414. doi:10.1038/gene.2011.21.
44. Galluzzi L, Yamazaki T, Kroemer G. Linking cellular stress responses to systemic homeostasis. Nat Rev Mol Cell Biol. 2018;19(11):731–745. doi:10.1038/s41580-018-0068-0.
45. Taniguchi K, Karin M. NF-kappaB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol. 2018;18:309–324.
46. Zhong Z, Sanchez-Lopez E, Karin M. Autophagy, inflammation, and immunity: a troika governing cancer and its treatment. Cell. 2016;166(2):288–298. doi:10.1016/j.cell.2016.05.051.

47. Sen GC, Sarkar SN. Transcriptional signaling by double-stranded RNA: role of TLR3. Cytokine Growth Factor Rev. 2005;16(1):1–14. doi:10.1016/j.cytogfr.2005.01.006.
48. Gosu V, Basith S, Kwon OP, Choi S. Therapeutic applications of nucleic acids and their analogues in Toll-like receptor signaling. Molecules. 2012;17(11):13503–13529. doi:10.3390/molecules171113503.
49. Sarkar SN, Smith HL, Rowe TM, Sen GC. Double-stranded RNA signaling by Toll-like receptor 3 requires specific tyrosine residues in its cytoplasmic domain. J Biol Chem. 2003;278(7):4393–4396. doi:10.1074/jbc.C200655200.
50. Wen AY, Sakamoto KM, Miller LS. The role of the transcription factor CREB in immune function. J Immunol. 2010;185(11):6413–6419. doi:10.4049/jimmunol.1001829.
51. Peroval MY, Boyd AC, Young JR, Smith AL. A critical role for MAPK signalling pathways in the transcriptional regulation of toll like receptors. PLoS One. 2013;8(2):e51243. doi:10.1371/journal.pone.0051243.
52. Campos PC, Gomes MT, Guimaraes ES, Guimaraes G, Oliveira SC. TLR7 and TLR3 sense brucella abortus RNA to induce proinflammatory cytokine production but they are dispensable for host control of infection. Front Immunol. 2017;8:28. doi:10.3389/fimmu.2017.00028.
53. Ryu JH, Park M, Kim BK, Ryu KH, Woo SY. Tonsil-derived mesenchymal stromal cells produce CXCR2-binding chemokines and acquire follicular dendritic cell-like phenotypes under TLR3 stimulation. Cytokine. 2015;73(2):225–235. doi:10.1016/j.cyto.2015.02.028.
54. Tan LSY, Wong B, Gangodu NR, Lee AZE, Kian Fong Liou A, KS L, Li H, Yann Lim M, Salazar AM, Lim CM, et al. Enhancing the immune stimulatory effects of cetuximab therapy through TLR3 signalling in Epstein-Barr virus (EBV) positive nasopharyngeal carcinoma. Oncoimmunology. 2018;7(11):e1500109. doi:10.1080/2162402X.2018.1500109.
55. Lai Y, Gallo RL. Toll-like receptors in skin infections and inflammatory diseases. Infect Disord Drug Targets. 2008;8(3):144–155. doi:10.2174/1871526510808030144.
56. Uematsu S, Akira S. Toll-like receptors and Type I interferons. J Biol Chem. 2007;282(21):15319–15323. doi:10.1074/jbc.R700009200.
57. Vacchelli E, Sistigu A, Yamazaki T, Vitale I, Zitvogel L, Kroemer G, Starmann J, Tjwa M, Plate KH, Sültmann H. Autocrine signaling of type 1 interferons in successful anticancer chemotherapy. Oncoimmunology. 2015;4(6):e988042. doi:10.1080/2162402X.2015.1008371.
58. Sarkar SN, Peters KL, Elco CP, Sakamoto S, Pal S, Sen GC. Novel roles of TLR3 tyrosine phosphorylation and PI3 kinase in double-stranded RNA signaling. Nat Struct Mol Biol. 2004;11(11):1060–1067. doi:10.1038/nsmb847.
59. Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science. 2003;301(5633):640–643. doi:10.1126/science.1087262.
60. Holtorf A, Conrad A, Holzmann B, Janssen KP. Cell-type specific MyD88 signaling is required for intestinal tumor initiation and progression to malignancy. Oncoimmunology. 2018;7:e1466770. doi:10.1080/2162402X.2018.1466770.
61. Gosu V, Son S, Shin D, Song KD. Insights into the dynamic nature of the dsRNA-bound TLR3 complex. Sci Rep. 2019;9(1):3652. doi:10.1038/s41598-019-39984-8.
62. Wang Y, Zhang S, Li H, Wang H, Zhang T, Hutchinson MR, Yin H, Wang X. Small-molecule modulators of toll-like receptors. Acc Chem Res. 2020. doi:10.1021/acs.accounts.9b00631.
63. Vacchelli E, Enot DP, Pietrocola F, Zitvogel L, Kroemer G. Impact of pattern recognition receptors on the prognosis of breast cancer patients undergoing adjuvant chemotherapy. Cancer Res. 2016;76(11):3122–3126. doi:10.1158/0008-5472.CAN-16-0294.
64. Vacchelli E, Ma Y, Baracco EE, Sistigu A, Enot DP, Pietrocola F, Yang H, Adjemian S, Chaba K, Semeraro M, et al. Chemotherapy-induced antitumor immunity requires formyl peptide receptor 1. Science. 2015;350(6263):972–978. doi:10.1126/science.aad0779.
65. Kong KF, Delroux K, Wang X, Qian F, Arjona A, Malawista SE, Fikrig E, Montgomery RR. Dysregulation of TLR3 impairs the innate immune response to West Nile virus in the elderly. J Virol. 2008;82(15):7613–7623. doi:10.1128/JVI.00618-08.
66. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, Vitale I, Goubar A, Baracco EE, Remédios C, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med. 2014;20(11):1301–1309. doi:10.1038/nm.3708.
67. Yeyeodu ST, Kidd LR, Oprea-Ilies GM, Burns BG, Vancleave TT, Shim JY, et al. IRAK4 and TLR3 sequence variants may alter breast cancer risk among African-Aamerican women. Front Immunol. 2013;4:338. doi:10.3389/fimmu.2013.00338.
68. Hasimu A, Ge L, Li QZ, Zhang RP, Guo X. Expressions of Toll-like receptors 3, 4, 7, and 9 in cervical lesions and their correlation with HPV16 infection in Uighur women. Chin J Cancer. 2011;30(5):344–350. doi:10.5732/cjc.010.10456.
69. Zeljic K, Supic G, Jovic N, Kozomara R, Brankovic-Magic M, Obrenovic M, Magic Z. Association of TLR2, TLR3, TLR4 and CD14 genes polymorphisms with oral cancer risk and survival. Oral Dis. 2014;20(4):416–424. doi:10.1111/odi.12144.
70. Li G, Zheng Z. Toll-like receptor 3 genetic variants and susceptibility to hepatocellular carcinoma and HBV-related hepatocellular carcinoma. Tumour Biol. 2013;34(3):1589–1594. doi:10.1007/s13277-013-0689-z.
71. Slattery ML, Herrick JS, Bondurant KL, Wolff RK. Toll-like receptor genes and their association with colon and rectal cancer development and prognosis. Int J Cancer. 2012;130(12):2974–2980. doi:10.1002/ijc.26314.
72. Castro FA, Forsti A, Buch S, Kalthoff H, Krauss C, Bauer M, Egberts J, Schniewind B, Broering DC, Schreiber S, et al. TLR-3 polymorphism is an independent prognostic marker for stage II colorectal cancer. Eur J Cancer. 2011;47(8):1203–1210. doi:10.1016/j.ejca.2010.12.011.
73. Dai J, Hu Z, Dong J, Xu L, Pan S, Jiang Y, Jin G, Chen Y, Shen H. Host immune gene polymorphisms were associated with the prognosis of non-small-cell lung cancer in Chinese. Int J Cancer. 2012;130(3):671–676. doi:10.1002/ijc.26067.
74. Chew V, Tow C, Huang C, Bard-Chapeau E, Copeland NG, Jenkins NA, Weber A, Lim KH, Toh HC, Heikenwalder M, et al. Toll-like receptor 3 expressing tumor parenchyma and infiltrating natural killer cells in hepatocellular carcinoma patients. J Natl Cancer Inst. 2012;104(23):1796–1807. doi:10.1093/jnci/djs436.
75. Yuan MM, Xu YY, Chen L, Li XY, Qin J, Shen Y. TLR3 expression correlates with apoptosis, proliferation and angiogenesis in hepatocellular carcinoma and predicts prognosis. BMC Cancer. 2015;15:245.
76. Hsu WM, Huang CC, Wu PY, Lee H, Huang MC, Tai MH, Chuang J-H. Toll-like receptor 3 expression inhibits cell invasion and migration and predicts a favorable prognosis in neuroblastoma. Cancer Lett. 2013;336(2):338–346. doi:10.1016/j.canlet.2013.03.024.
77. Salaun B, Zitvogel L, Asselin-Paturel C, Morel Y, Chemin K, Dubois C, Massacrier C, Conforti R, Chenard MP, Sabourin J-C, et al. TLR3 as a biomarker for the therapeutic efficacy of double-stranded RNA in breast cancer. Cancer Res. 2011;71(5):1607–1614. doi:10.1158/0008-5472.CAN-10-3490.
78. Deutsch E, Chargari C, Galluzzi L, Kroemer G. Optimising efficacy and reducing toxicity of anticancer radioimmunotherapy. Lancet Oncol. 2019;20(8):e452–e63. doi:10.1016/S1470-2045(19)30171-8.
79. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. Nat Rev Immunol. 2017;17(2):97–111. doi:10.1038/nri.2016.107.
80. Galluzzi L, Vitale I, Warren S, Adjemian S, Agostinis P, Martinez AB. Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. J Immunother Cancer. 2020;88(1):e000337.

81. Garg AD, Agostinis P. Cell death and immunity in cancer: from danger signals to mimicry of pathogen defense responses. Immunol Rev. 2017;280(1):126–148. doi:10.1111/imr.12574.
82. Vacchelli E, Ma Y, Baracco EE, Zitvogel L, Kroemer G. Yet another pattern recognition receptor involved in the chemotherapy-induced anticancer immune response: formyl peptide receptor-1. Oncoimmunology. 2016;5(5):e1118600. doi:10.1080/2162402X.2015.1118600.
83. Galluzzi L, Chan TA, Kroemer G, Wolchok JD, Lopez-Soto A. The hallmarks of successful anticancer immunotherapy. Sci Transl Med. 2018;10(459):eaat7807.
84. Medler T, Patel JM, Alice A, Baird JR, Hu HM, Gough MJ. Activating the nucleic acid-sensing machinery for anticancer immunity. Int Rev Cell Mol Biol. 2019;344:173–214.
85. Pastor F, Berraondo P, Etxeberria I, Frederick J, Sahin U, Gilboa E, Melero I. An RNA toolbox for cancer immunotherapy. Nat Rev Drug Discov. 2018;17(10):751–767. doi:10.1038/nrd.2018.132.
86. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. Nat Rev Immunol. 2020;20(1):7–24. doi:10.1038/s41577-019-0210-z.
87. Hennessy EJ, Parker AE, O'Neill LA. Targeting Toll-like receptors: emerging therapeutics? Nat Rev Drug Discov. 2010;9(4):293–307. doi:10.1038/nrd3203.
88. Vacchelli E, Galluzzi L, Eggermont A, Fridman WH, Galon J, Sautes-Fridman C. Trial watch: FDA-approved Toll-like receptor agonists for cancer therapy. Oncoimmunology. 2012;1:894–907.
89. Bezu L, Kepp O, Cerrato G, Pol J, Fucikova J, Spisek R, Zitvogel L, Kroemer G, Galluzzi L. Trial watch: peptide-based vaccines in anticancer therapy. Oncoimmunology. 2018;7(12):e1511506. doi:10.1080/2162402X.2018.1511506.
90. Smith M, Garcia-Martinez E, Pitter MR, Fucikova J, Spisek R, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: toll-like receptor agonists in cancer immunotherapy. Oncoimmunology. 2018;7(12):e1526250. doi:10.1080/2162402X.2018.1526250.
91. Maisonneuve C, Bertholet S, Philpott DJ, De Gregorio E. Unleashing the potential of NOD- and Toll-like agonists as vaccine adjuvants. Proc Natl Acad Sci U S A. 2014;111(34):12294–12299. doi:10.1073/pnas.1400478111.
92. Marshall-Clarke S, Downes JE, Haga IR, Bowie AG, Borrow P, Pennock JL, Grencis RK, Rothwell P. Polyinosinic acid is a ligand for toll-like receptor 3. J Biol Chem. 2007;282(34):24759–24766. doi:10.1074/jbc.M700188200.
93. Sugiyama T, Hoshino K, Saito M, Yano T, Sasaki I, Yamazaki C, Akira S, Kaisho T. Immunoadjuvant effects of polyadenylic: polyuridylicacids through TLR3 and TLR7. Int Immunol. 2008;20(1):1–9. doi:10.1093/intimm/dxm112.
94. Salazar AM, Erlich RB, Mark A, Bhardwaj N, Herberman RB. Therapeutic in situ autovaccination against solid cancers with intratumoral poly-ICLC: case report, hypothesis, and clinical trial. Cancer Immunol Res. 2014;2(8):720–724. doi:10.1158/2326-6066.CIR-14-0024.
95. Brodsky I, Strayer DR, Krueger LJ, Carter WA. Clinical studies with ampligen (mismatched double-stranded RNA). J Biol Response Mod. 1985;4:669–675.
96. Navabi H, Jasani B, Reece A, Clayton A, Tabi Z, Donninger C, Mason M, Adams M. A clinical grade poly I:C-analogue (Ampligen) promotes optimal DC maturation and Th1-type T cell responses of healthy donors and cancer patients in vitro. Vaccine. 2009;27(1):107–115. doi:10.1016/j.vaccine.2008.10.024.
97. Berk E, Kalinski P. Lymphocyte-polarized DC1s: effective inducers of tumor-specific CTLs. Oncoimmunology. 2012;1(8):1443–1444. doi:10.4161/onci.21295.
98. Bianchi F, Pretto S, Tagliabue E, Balsari A, Sfondrini L. Exploiting poly(I:C) to induce cancer cell apoptosis. Cancer Biol Ther. 2017;18(10):747–756. doi:10.1080/15384047.2017.1373220.
99. Conforti R, Ma Y, Morel Y, Paturel C, Terme M, Viaud S, Ryffel B, Ferrantini M, Uppaluri R, Schreiber R, et al. Opposing effects of toll-like receptor (TLR3) signaling in tumors can be therapeutically uncoupled to optimize the anticancer efficacy of TLR3 ligands. Cancer Res. 2010;70(2):490–500. doi:10.1158/0008-5472.CAN-09-1890.
100. Nicodemus CF, Berek JS. TLR3 agonists as immunotherapeutic agents. Immunotherapy. 2010;2(2):137–140. doi:10.2217/imt.10.8.
101. Sharma S, Zhu L, Davoodi M, Harris-White M, Lee JM, St John M, Salgia R, Dubinett S. TLR3 agonists and proinflammatory antitumor activities. Expert Opin Ther Targets. 2013;17(5):481–483. doi:10.1517/14728222.2013.781585.
102. Adams S. Toll-like receptor agonists in cancer therapy. Immunotherapy. 2009;1(6):949–964. doi:10.2217/imt.09.70.
103. Aranda F, Vacchelli E, Obrist F, Eggermont A, Galon J, Sautes-Fridman C, Cremer I, Henrik Ter Meulen J, Zitvogel L, Kroemer G, et al. Trial Watch: toll-like receptor agonists in oncological indications. Oncoimmunology. 2014;3(6):e29179. doi:10.4161/onci.29179.
104. Devaud C, John LB, Westwood JA, Darcy PK, Kershaw MH. Immune modulation of the tumor microenvironment for enhancing cancer immunotherapy. Oncoimmunology. 2013;2(8):e25961. doi:10.4161/onci.25961.
105. Lu L, Barbi J, Pan F. The regulation of immune tolerance by FOXP3. Nat Rev Immunol. 2017;17(11):703–717. doi:10.1038/nri.2017.75.
105. Togashi Y, Shitara K, Nishikawa H. Regulatory T cells in cancer immunosuppression - implications for anticancer therapy. Nat Rev Clin Oncol. 2019;16(6):356–371. doi:10.1038/s41571-019-0175-7.
106. O'Donnell JS, Teng MWL, Smyth MJ. Cancer immunoediting and resistance to T cell-based immunotherapy. Nat Rev Clin Oncol. 2019;16(3):151–167. doi:10.1038/s41571-018-0142-8.
107. Rodriguez-Ruiz ME, Vitale I, Harrington KJ, Melero I, Galluzzi L. Immunological impact of cell death signaling driven by radiation on the tumor microenvironment. Nat Immunol. 2020;21(2):120–134. doi:10.1038/s41590-019-0561-4.
108. Field AK, Tytell AA, Lampson GP, Hilleman MR. Inducers of interferon and host resistance. II. Multistranded synthetic polynucleotide complexes. Proc Natl Acad Sci U S A. 1967;58(3):1004–1010. doi:10.1073/pnas.58.3.1004.
109. Yarchoan M, Johnson BA 3rd, Lutz ER, Laheru DA, Jaffee EM. Targeting neoantigens to augment antitumour immunity. Nat Rev Cancer. 2017;17(4):209–222. doi:10.1038/nrc.2016.154.
109. Verdijk RM, Mutis T, Esendam B, Kamp J, Melief CJ, Brand A, Goulmy E. Polyriboinosinic polyribocytidylic acid (poly(I:C)) induces stable maturation of functionally active human dendritic cells. J Immunol. 1999;163:57–61.
110. Jelinek I, Leonard JN, Price GE, Brown KN, Meyer-Manlapat A, Goldsmith PK, Wang Y, Venzon D, Epstein SL, Segal DM, et al. TLR3-specific double-stranded RNA oligonucleotide adjuvants induce dendritic cell cross-presentation, CTL responses, and antiviral protection. J Immunol. 2011;186(4):2422–2429. doi:10.4049/jimmunol.1002845.
111. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, Pandey G, Leboeuf M, Elpek KG, Helft J, et al. Deciphering the transcriptional network of the dendritic cell lineage. Nat Immunol. 2012;13(9):888–899. doi:10.1038/ni.2370.
112. Roselli E, Araya P, Nunez NG, Gatti G, Graziano F, Sedlik C, Benaroch P, Piaggio E, Maccioni M. TLR3 activation of intratumoral CD103+ dendritic cells modifies the tumor infiltrate conferring anti-tumor immunity. Front Immunol. 2019;10:503. doi:10.3389/fimmu.2019.00503.
113. Bachem A, Guttler S, Hartung E, Ebstein F, Schaefer M, Tannert A, Salama A, Movassaghi K, Opitz C, Mages HW, et al. Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. J Exp Med. 2010;207(6):1273–1281. doi:10.1084/jem.20100348.
114. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, Chen CJJ, Dunbar PR, Wadley RB, Jeet V, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. J Exp Med. 2010;207(6):1247–1260. doi:10.1084/jem.20092140.

115. Balan S, Saxena M, Bhardwaj N. Dendritic cell subsets and locations. Int Rev Cell Mol Biol. 2019;348:1–68.
116. Eisenbarth SC. Dendritic cell subsets in T cell programming: location dictates function. Nat Rev Immunol. 2019;19(2):89–103. doi:10.1038/s41577-018-0088-1.
117. Lee YS, Radford KJ. The role of dendritic cells in cancer. Int Rev Cell Mol Biol. 2019;348:123–178.
118. Spranger S, Dai D, Horton B, Gajewski TF. Tumor-residing Batf3 dendritic cells are required for effector T cell trafficking and adoptive T cell therapy. Cancer Cell. 2017;31(5):711–23 e4. doi:10.1016/j.ccell.2017.04.003.
119. Corrales L, Matson V, Flood B, Spranger S, Gajewski TF. Innate immune signaling and regulation in cancer immunotherapy. Cell Res. 2017;27(1):96–108. doi:10.1038/cr.2016.149.
120. Kotsias F, Cebrian I, Alloatti A. Antigen processing and presentation. Int Rev Cell Mol Biol. 2019;348:69–121.
121. Kline DE, MacNabb BW, Chen X, Chan WC, Fosco D, Kline J. CD8α+dendritic cells dictate leukemia-specific CD8+T cell fates. J Immunol. 2018;201(12):3759–3769. doi:10.4049/jimmunol.1801184.
122. Guinn ZP, Petro TM. IFN-gamma synergism with poly I:C reduces growth of murine and human cancer cells with simultaneous changes in cell cycle and immune checkpoint proteins. Cancer Lett. 2018;438:1–9. doi:10.1016/j.canlet.2018.09.003.
123. Salmon H, Idoyaga J, Rahman A, Leboeuf M, Remark R, Jordan S, Casanova-Acebes M, Khudoynazarova M, Agudo J, Tung N, et al. Expansion and activation of CD103 + dendritic cell progenitors at the tumor site enhances tumor responses to therapeutic PD-L1 and BRAF inhibition. Immunity. 2016;44(4):924–938. doi:10.1016/j.immuni.2016.03.012.
124. Di S, Zhou M, Pan Z, Sun R, Chen M, Jiang H. Combined adjuvant of poly I:C improves antitumor effects of CAR-T Cells. Front Oncol. 2019;9:241. doi:10.3389/fonc.2019.00241.
125. Zhao J, Xue Y, Pan Y, Yao A, Wang G, Li D, Wang T, Zhao S, Hou Y. Toll-like receptor 3 agonist poly I:C reinforces the potency of cytotoxic chemotherapy via the TLR3-UNC93B1-IFN-beta signaling axis in paclitaxel-resistant colon cancer. J Cell Physiol. 2019;234(5):7051–7061. doi:10.1002/jcp.27459.
126. Zitvogel L, Kroemer G. CD103+ dendritic cells producing interleukin-12 in anticancer immunosurveillance. Cancer Cell. 2014;26(5):591–593. doi:10.1016/j.ccell.2014.10.008.
127. Whilding LM, Maher J. ErbB-targeted CAR T-cell immunotherapy of cancer. Immunotherapy. 2015;7(3):229–241. doi:10.2217/imt.14.120.
128. Alqahtani FY, Aleanizy FS, El Tahir E, Alkahtani HM, AlQuadeib BT. Paclitaxel. Profiles Drug Subst Excip Relat Methodol. 2019;44:205–238.
129. Vanpouille-Box C, Demaria S, Formenti SC, Galluzzi L. Cytosolic DNA sensing in organismal tumor control. Cancer Cell. 2018;34(3):361–378. doi:10.1016/j.ccell.2018.05.013.
130. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P. Molecular mechanisms of cell death: recommendations of the nomenclature committee on cell death 2018. Cell Death Differ. 2018;25:486–541.
131. Kaiser WJ, Offermann MK. Apoptosis induced by the toll-like receptor adaptor TRIF is dependent on its receptor interacting protein homotypic interaction motif. J Immunol. 2005;174(8):4942–4952. doi:10.4049/jimmunol.174.8.4942.
132. Conrad M, Angeli JP, Vandenabeele P, Stockwell BR. Regulated necrosis: disease relevance and therapeutic opportunities. Nat Rev Drug Discov. 2016;15(5):348–366. doi:10.1038/nrd.2015.6.
133. Kaiser WJ, Sridharan H, Huang C, Mandal P, Upton JW, Gough PJ, Sehon CA, Marquis RW, Bertin J, Mocarski ES, et al. Toll-like receptor 3-mediated necrosis via TRIF, RIP3, and MLKL. J Biol Chem. 2013;288(43):31268–31279. doi:10.1074/jbc.M113.462341.
134. Weinlich R, Oberst A, Beere HM, Green DR. Necroptosis in development, inflammation and disease. Nat Rev Mol Cell Biol. 2017;18(2):127–136. doi:10.1038/nrm.2016.149.
135. Galluzzi L, Kepp O, Chan FK, Kroemer G. Necroptosis: mechanisms and relevance to disease. Annu Rev Pathol. 2017;12(1):103–130. doi:10.1146/annurev-pathol-052016-100247.
136. Takemura R, Takaki H, Okada S, Shime H, Akazawa T, Oshiumi H, Matsumoto M, Teshima T, Seya T. PolyI:C-Induced, TLR3/RIP3-dependent necroptosis backs up immune effector-mediated tumor elimination in vivo. Cancer Immunol Res. 2015;3(8):902–914. doi:10.1158/2326-6066.CIR-14-0219.
137. Aznar MA, Planelles L, Perez-Olivares M, Molina C, Garasa S, Etxeberria I, Perez G, Rodriguez I, Bolaños E, Lopez-Casas P, et al. Immunotherapeutic effects of intratumoral nanoplexed poly I:C. J Immunother Cancer. 2019;7(1):116. doi:10.1186/s40425-019-0568-2.
138. Palchetti S, Starace D, De Cesaris P, Filippini A, Ziparo E, Riccioli A. Transfected poly(I:C) activates different dsRNA receptors, leading to apoptosis or immunoadjuvant response in androgen-independent prostate cancer cells. J Biol Chem. 2015;290(9):5470–5483. doi:10.1074/jbc.M114.601625.
139. Song H, Huang P, Niu J, Shi G, Zhang C, Kong D, Wang W. Injectable polypeptide hydrogel for dual-delivery of antigen and TLR3 agonist to modulate dendritic cells in vivo and enhance potent cytotoxic T-lymphocyte response against melanoma. Biomaterials. 2018;159:119–129. doi:10.1016/j.biomaterials.2018.01.004.
140. Lacour J, Lacour F, Spira A, Michelson M, Petit JY, Delage G, Delage G, Contesso G, Viguier J. Adjuvant treatment with polyadenylic-polyuridylic acid (Polya.Polyu) in operable breast cancer. Lancet. 1980;2(8187):161–164. doi:10.1016/S0140-6736(80)90057-4.
141. Lacour J, Lacour F, Spira A, Michelson M, Petit JY, Delage G, Sarrazin D, Contesso G, Viguier J. Adjuvant treatment with polyadenylic-polyuridylic acid in operable breast cancer: updated results of a randomised trial. Br Med J (Clin Res Ed). 1984;288(6417):589–592. doi:10.1136/bmj.288.6417.589.
142. Ducret JP, Caille P, Sancho Garnier H, Amiel JL, Michelson M, Hovanessian AG, Youn JK, Lacour F. A phase I clinical tolerance study of polyadenylic-polyuridylic acid in cancer patients. J Biol Response Mod. 1985;4:129–133.
142. Levine AS, Levy HB. Phase I-II trials of poly IC stabilized with poly-L-lysine. Cancer Treat Rep. 1978;62:1907–1912.
143. Robinson RA, DeVita VT, Levy HB, Baron S, Hubbard SP, Levine AS. A phase I-II trial of multiple-dose polyriboinosic-polyribocytidylic acid in patieonts with leukemia or solid tumors. J Natl Cancer Inst. 1976;57(3):599–602. doi:10.1093/jnci/57.3.599.
144. Perrot I, Deauvieau F, Massacrier C, Hughes N, Garrone P, Durand I, Demaria O, Viaud N, Gauthier L, Blery M, et al. TLR3 and Rig-like receptor on myeloid dendritic cells and Rig-like receptor on human NK cells are both mandatory for production of IFN-gamma in response to double-stranded RNA. J Immunol. 2010;185(4):2080–2088. doi:10.4049/jimmunol.1000532.
145. Nowacki TM, Kuerten S, Zhang W, Shive CL, Kreher CR, Boehm BO, Lehmann PV, Tary-Lehmann M. Granzyme B production distinguishes recently activated CD8(+) memory cells from resting memory cells. Cell Immunol. 2007;247(1):36–48. doi:10.1016/j.cellimm.2007.07.004.
146. Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. Nat Rev Immunol. 2018;18(3):153–167. doi:10.1038/nri.2017.108.
147. Park SJ, Ye W, Xiao R, Silvin C, Padget M, Hodge JW, Van Waes C, Schmitt NC. Cisplatin and oxaliplatin induce similar immunogenic changes in preclinical models of head and neck cancer. Oral Oncol. 2019;95:127–135. doi:10.1016/j.oraloncology.2019.06.016.
148. Yamazaki T, Buque A, Ames TD, Galluzzi L. PT-112 induces immunogenic cell death and synergizes with immune checkpoint blockers in mouse tumor models. Oncoimmunology. 2020;9(1):1721810. doi:10.1080/2162402X.2020.1721810.
149. Rao S, Gharib K, Han A. Cancer Immunosurveillance by T Cells. Int Rev Cell Mol Biol. 2019;342:149–173.

150. Sharma P, Allison JP. Dissecting the mechanisms of immune checkpoint therapy. Nat Rev Immunol. 2020;20(2):75–76. doi:10.1038/s41577-020-0275-8.
151. Jasani B, Navabi H, Adams M. Ampligen: a potential toll-like 3 receptor adjuvant for immunotherapy of cancer. Vaccine. 2009;27 (25–26):3401–3404. doi:10.1016/j.vaccine.2009.01.071.
152. Strayer DR, Carter WA, Strauss KI, Brodsky I, Suhadolnik R, Ablashi O, Henry B, Mitchell WM, Bastien S, Peterson D, et al. Long term improvements in patients with chronic fatigue syndrome treated with ampligen. J Chronic Fatigue Syndr. 1995;1 (1):35–53. doi:10.1300/J092v01n01_04.
153. Strayer DR, Carter WA, Stouch BC, Stevens SR, Bateman L, Cimoch PJ, Lapp CW, Peterson DL, Mitchell WM. A double-blind, placebo-controlled, randomized, clinical trial of the TLR-3 agonist rintatolimod in severe cases of chronic fatigue syndrome. PLoS One. 2012;7(3):e31334. doi:10.1371/journal.pone.0031334.
154. Killock D. Haematological cancer: resiquimod-a topical CTCL therapy. Nat Rev Clin Oncol. 2015;12(10):563. doi:10.1038/nrclinonc.2015.142.
155. Nishii N, Tachinami H, Kondo Y, Xia Y, Kashima Y, Ohno T, Nagai S, Li L, Lau W, Harada H, et al. Systemic administration of a TLR7 agonist attenuates regulatory T cells by dendritic cell modification and overcomes resistance to PD-L1 blockade therapy. Oncotarget. 2018;9(17):13301–13312. doi:10.18632/oncotarget.24327.
156. Gutierrez C, Schiff R. HER2: biology, detection, and clinical implications. Arch Pathol Lab Med. 2011;135(1):55–62. doi:10.1043/2010-0454-RAR.1.
157. Nath S, Mukherjee P. MUC1: a multifaceted oncoprotein with a key role in cancer progression. Trends Mol Med. 2014;20 (6):332–342. doi:10.1016/j.molmed.2014.02.007.
158. Tomasicchio M, Semple L, Esmail A, Meldau R, Randall P, Pooran A, Davids M, Cairncross L, Anderson D, Downs J, et al. An autologous dendritic cell vaccine polarizes a Th-1 response which is tumoricidal to patient-derived breast cancer cells. Cancer Immunol Immunother. 2019;68(1):71–83. doi:10.1007/s00262-018-2238-5.
159. Naumann K, Wehner R, Schwarze A, Petzold C, Schmitz M, Rohayem J. Activation of dendritic cells by the novel Toll-like receptor 3 agonist RGC100. Clin Dev Immunol. 2013;2013:283649. doi:10.1155/2013/283649.
160. Jain A, Barve A, Zhao Z, Jin W, Cheng K. Comparison of avidin, neutravidin, and streptavidin as nanocarriers for efficient siRNA delivery. Mol Pharm. 2017;14(5):1517–1527. doi:10.1021/acs.molpharmaceut.6b00933.
161. Vermette P, Gengenbach T, Divisekera U, Kambouris PA, Griesser HJ, Meagher L. Immobilization and surface characterization of NeutrAvidin biotin-binding protein on different hydrogel interlayers. J Colloid Interface Sci. 2003;259(1):13–26. doi:10.1016/S0021-9797(02)00185-6.
162. Schau I, Michen S, Hagstotz A, Janke A, Schackert G, Appelhans D, Temme A. Targeted delivery of TLR3 agonist to tumor cells with single chain antibody fragment-conjugated nanoparticles induces type I-interferon response and apoptosis. Sci Rep. 2019;9(1):3299. doi:10.1038/s41598-019-40032-8.
163. Matsumoto M, Tatematsu M, Nishikawa F, Azuma M, Ishii N, Morii-Sakai A, Shime H, Seya T. Defined TLR3-specific adjuvant that induces NK and CTL activation without significant cytokine production in vivo. Nat Commun. 2015;6(1):6280. doi:10.1038/ncomms7280.
164. Khodarev NN. Intracellular RNA sensing in mammalian cells: role in stress response and cancer therapies. Int Rev Cell Mol Biol. 2019;344:31–89.
165. Rehwinkel J, Gack MU. RIG-I-like receptors: their regulation and roles in RNA sensing. Nat Rev Immunol. 2020. doi:10.1038/s41577-020-0288-3.
166. Matsumoto M, Takeda Y, Seya T. Targeting Toll-like receptor 3 in dendritic cells for cancer immunotherapy. Expert Opin Biol Ther. 2020;1–10. doi:10.1080/14712598.2020.1749260.
167. Seya T, Takeda Y, Matsumoto M. Tumor vaccines with dsRNA adjuvant ARNAX induces antigen-specific tumor shrinkage without cytokinemia. Oncoimmunology. 2016;5(2):e1043506. doi:10.1080/2162402X.2015.1043506.
168. Seya T, Takeda Y, Takashima K, Yoshida S, Azuma M, Matsumoto M. Adjuvant immunotherapy for cancer: both dendritic cell-priming and check-point inhibitor blockade are required for immunotherapy. Proc Jpn Acad Ser B Phys Biol Sci. 2018;94 (3):153–160. doi:10.2183/pjab.94.011.
169. Takeda Y, Yoshida S, Takashima K, Ishii-Mugikura N, Shime H, Seya T, Matsumoto M. Vaccine immunotherapy with ARNAX induces tumor-specific memory T cells and durable anti-tumor immunity in mouse models. Cancer Sci. 2018;109(7):2119–2129. doi:10.1111/cas.13649.
170. Bianchi F, Milione M, Casalini P, Centonze G, Le Noci VM, Storti C, Alexiadis S, Truini M, Sozzi G, Pastorino U, et al. Toll-like receptor 3 as a new marker to detect high risk early stage non-small-cell lung cancer patients. Sci Rep. 2019;9(1):14288. doi:10.1038/s41598-019-50756-2.
171. Galluzzi L, Lopez-Soto A, Kumar S, Kroemer G. Caspases connect cell-death signaling to organismal homeostasis. Immunity. 2016;44 (2):221–231. doi:10.1016/j.immuni.2016.01.020.
172. Man SM, Kanneganti TD. Converging roles of caspases in inflammasome activation, cell death and innate immunity. Nat Rev Immunol. 2016;16(1):7–21. doi:10.1038/nri.2015.7.
173. Rodriguez-Ruiz ME, Buque A, Hensler M, Chen J, Bloy N, Petroni G, Sato A, Yamazaki T, Fucikova J, Galluzzi L, et al. Apoptotic caspases inhibit abscopal responses to radiation and identify a new prognostic biomarker for breast cancer patients. Oncoimmunology. 2019;8(11):e1655964. doi:10.1080/2162402X.2019.1655964.
174. Hammerich L, Marron TU, Upadhyay R, Svensson-Arvelund J, Dhainaut M, Hussein S. Systemic clinical tumor regressions and potentiation of PD1 blockade with in situ vaccination. Nat Med. 2019;25(5):814–824. doi:10.1038/s41591-019-0410-x.
175. Hassel JC, Jiang H, Bender C, Winkler J, Sevko A, Shevchenko I, Halama N, Dimitrakopoulou-Strauss A, Haefeli WE, Jäger D, et al. Tadalafil has biologic activity in human melanoma. Results of a pilot trial with Ta dalafil in patients with metastatic Melanoma (TaMe). Oncoimmunology. 2017;6(9):e1326440. doi:10.1080/2162402X.2017.1326440.
176. Weed DT, Zilio S, Reis IM, Sargi Z, Abouyared M, Gomez-Fernandez CR, Civantos FJ, Rodriguez CP, Serafini P. The reversal of immune exclusion mediated by tadalafil and an anti-tumor vaccine also induces PDL1 upregulation in recurrent head and neck squamous cell carcinoma: interim analysis of a phase I clinical trial. Front Immunol. 2019;10:1206. doi:10.3389/fimmu.2019.01206.
177. Kyi C, Roudko V, Sabado R, Saenger Y, Loging W, Mandeli J, Thin TH, Lehrer D, Donovan M, Posner M, et al. Therapeutic immune modulation against solid cancers with intratumoral poly-ICLC: a pilot trial. Clin Cancer Res. 2018;24(20):4937–4948. doi:10.1158/1078-0432.CCR-17-1866.
178. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, Ly A, Lie W-R, Hildebrand WH, Mardis ER, et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science. 2015;348(6236):803–808. doi:10.1126/science.aaa3828.
179. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, Zhang W, Luoma A, Giobbie-Hurder A, Peter L, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature. 2017;547(7662):217–221. doi:10.1038/nature22991.
180. Sahin U, Derhovanessian E, Miller M, BP K, Simon P, Lower M, Bukur V, Tadmor AD, Luxemburger U, Schrörs B, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature. 2017;547(7662):222–226. doi:10.1038/nature23003.
181. Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, Oliveira G, Giobbie-Hurder A, Felt K, Gjini E, et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib

glioblastoma trial. Nature. 2019;565(7738):234–239. doi:10.1038/s41586-018-0792-9.
182. Rutledge WC, Kong J, Gao J, Gutman DA, Cooper LA, Appin C. Tumor-infiltrating lymphocytes in glioblastoma are associated with specific genomic alterations and related to transcriptional class. Clin Cancer Res. 2013;19(18):4951–4960. doi:10.1158/1078-0432.CCR-13-0551.
183. Johanns TM, Miller CA, Liu CJ, Perrin RJ, Bender D, Kobayashi DK, Campian JL, Chicoine MR, Dacey RG, Huang J, et al. Detection of neoantigen-specific T cells following a personalized vaccine in a patient with glioblastoma. Oncoimmunology. 2019;8(4):e1561106. doi:10.1080/2162402X.2018.1561106.
184. Ferrara N, Adamis AP. Ten years of anti-vascular endothelial growth factor therapy. Nat Rev Drug Discov. 2016;15(6):385–403. doi:10.1038/nrd.2015.17.
185. Kazazi-Hyseni F, Beijnen JH, Schellens JH. Bevacizumab. Oncologist. 2010;15(8):819–825. doi:10.1634/theoncologist.2009-0317.
186. Boydell E, Marinari E, Migliorini D, Dietrich PY, Patrikidou A, Dutoit V. Exploratory study of the effect of IMA950/poly-ICLC vaccination on response to bevacizumab in relapsing high-grade glioma patients. Cancers (Basel). 2019;11(4):464.
187. Slingluff CL Jr., Petroni GR, Chianese-Bullock KA, Smolkin ME, Hibbitts S, Murphy C, Johansen N, Grosh WW, Yamshchikov GV, Neese PY, et al. Immunologic and clinical outcomes of a randomized phase II trial of two multipeptide vaccines for melanoma in the adjuvant setting. Clin Cancer Res. 2007;13(21):6386–6395. doi:10.1158/1078-0432.CCR-07-0486.
188. Slingluff CL Jr., Petroni GR, Chianese-Bullock KA, Smolkin ME, Ross MI, Haas NB. Randomized multicenter trial of the effects of melanoma-associated helper peptides and cyclophosphamide on the immunogenicity of a multipeptide melanoma vaccine. J Clin Oncol. 2011;29(21):2924–2932. doi:10.1200/JCO.2010.33.8053.
189. Eaton MD, Scala AR. Further observations on the inhibitory effect of myxoviruses on a transplantable murine leukemia. Proc Soc Exp Biol Med. 1969;132(1):20–26. doi:10.3181/00379727-132-34138.
190. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, Takeda K, Akira S. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity. 1999;11(4):443–451. doi:10.1016/S1074-7613(00)80119-3.
191. Melssen MM, Petroni GR, Chianese-Bullock KA, Wages NA, Grosh WW, Varhegyi N. A multipeptide vaccine plus toll-like receptor agonists LPS or polyICLC in combination with incomplete Freund's adjuvant in melanoma patients. J Immunother Cancer. 2019;7(1):163. doi:10.1186/s40425-019-0625-x.
192. Aguilera D, MacDonald T, Castellino R, Janss A, Mazewski C, Kadom N, Pu M, Messer K, Crawford J, Connelly E, et al. PDCT-03. A phase III trial of poly-ICLC in the management of recurrent or progressive pediatric low grade gliomas. Results for the neurofibromatosis 1 group. (NCT01188096). Neuro-Oncology. 2018;20(suppl_6):vi201. doi:10.1093/neuonc/noy148.833.
193. Ammi R, De Waele J, Willemen Y, Van Brussel I, Schrijvers DM, Lion E. Poly(I:C) as cancer vaccine adjuvant: knocking on the door of medical breakthroughs. Pharmacol Ther. 2015;146:120–131. doi:10.1016/j.pharmthera.2014.09.010.
194. Levy HB, Baer G, Baron S, Buckler CE, Gibbs CJ, Iadarola MJ. A modified polyriboinosinic-polyribocytidylic acid complex that induces interferon in primates. J Infect Dis. 1975;132:434–439.
195. Rutz S, Wang X, Ouyang W. The IL-20 subfamily of cytokines–from host defence to tissue homeostasis. Nat Rev Immunol. 2014;14(12):783–795. doi:10.1038/nri3766.
196. Kuryk L, Moller AW, Jaderberg M. Combination of immunogenic oncolytic adenovirus ONCOS-102 with anti-PD-1 pembrolizumab exhibits synergistic antitumor effect in humanized A2058 melanoma huNOG mouse model. Oncoimmunology. 2019;8(2):e1532763. doi:10.1080/2162402X.2018.1532763.
197. Kroemer G, Galluzzi L. Combinatorial immunotherapy with checkpoint blockers solves the problem of metastatic melanoma-An exclamation sign with a question mark. Oncoimmunology. 2015;4(7):e1058037. doi:10.1080/2162402X.2015.1058037.
198. Parmar A, Chan KKW, Ko YJ. Metastatic colorectal cancer: therapeutic options for treating refractory disease. Curr Oncol. 2019;26(1):S24–S32. doi:10.3747/co.26.5575.
199. Chow FC, Chok KS. Colorectal liver metastases: an update on multidisciplinary approach. World J Hepatol. 2019;11(2):150–172. doi:10.4254/wjh.v11.i2.150.
200. Kokoschka EM, Trautinger F, Knobler RM, Pohl-Markl H, Micksche M. Long-term adjuvant therapy of high-risk malignant melanoma with interferon alpha 2b. J Invest Dermatol. 1990;95(6):193S–7S. doi:10.1111/1523-1747.ep12875517.
201. Li Y, Fang M, Zhang J, Wang J, Song Y, Shi J, Li W, Wu G, Ren J, Wang Z, et al. Hydrogel dual delivered celecoxib and anti-PD-1 synergistically improve antitumor immunity. Oncoimmunology. 2016;5(2):e1074374. doi:10.1080/2162402X.2015.1074374.
202. Semeraro M, Adam J, Stoll G, Louvet E, Chaba K, Poirier-Colame V. The ratio of CD8+/FOXP3 T lymphocytes infiltrating breast tissues predicts the relapse of ductal carcinoma in situ. Oncoimmunology. 2016;5(10):e1218106. doi:10.1080/2162402X.2016.1218106.
203. Senovilla L, Vitale I, Martins I, Tailler M, Pailleret C, Michaud M, Galluzzi L, Adjemian S, Kepp O, Niso-Santano M, et al. An immunosurveillance mechanism controls cancer cell ploidy. Science. 2012;337(6102):1678–1684. doi:10.1126/science.1224922.
204. Fridman WH, Zitvogel L, Sautes-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol. 2017;14(12):717–734. doi:10.1038/nrclinonc.2017.101.
205. Motzer RJ, Tannir NM, McDermott DF, Aren Frontera O, Melichar B, Choueiri TK. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. N Engl J Med. 2018;378(14):1277–1290. doi:10.1056/NEJMoa1712126.
206. Hellmann MD, Ciuleanu TE, Pluzanski A, Lee JS, Otterson GA, Audigier-Valette C. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med. 2018;378(22):2093–2104. doi:10.1056/NEJMoa1801946.
207. Kverneland AH, Enevold C, Donia M, Bastholt L, Svane IM, Nielsen CH. Development of anti-drug antibodies is associated with shortened survival in patients with metastatic melanoma treated with ipilimumab. Oncoimmunology. 2018;7(5):e1424674. doi:10.1080/2162402X.2018.1424674.
208. Madonna G, Ballesteros-Merino C, Feng Z, Bifulco C, Capone M, Giannarelli D. PD-L1 expression with immune-infiltrate evaluation and outcome prediction in melanoma patients treated with ipilimumab. Oncoimmunology. 2018;7:e1405206. doi:10.1080/2162402X.2017.1405206.
209. Wu X, Giobbie-Hurder A, Connolly EM, Li J, Liao X, Severgnini M, Zhou J, Rodig S, Hodi FS. Anti-CTLA-4 based therapy elicits humoral immunity to galectin-3 in patients with metastatic melanoma. Oncoimmunology. 2018;7(7):e1440930. doi:10.1080/2162402X.2018.1440930.
210. Castoldi F, Pietrocola F, Maiuri MC, Kroemer G. Aspirin induces autophagy via inhibition of the acetyltransferase EP300. Oncotarget. 2018;9(37):24574–24575. doi:10.18632/oncotarget.25364.
211. Pietrocola F, Castoldi F, Maiuri MC, Kroemer G. Aspirin-another caloric-restriction mimetic. Autophagy. 2018;14(7):1162–1163. doi:10.1080/15548627.2018.1454810.
212. Pietrocola F, Castoldi F, Markaki M, Lachkar S, Chen G, Enot DP. Aspirin recapitulates features of caloric restriction. Cell Rep. 2018;22(9):2395–2407. doi:10.1016/j.celrep.2018.02.024.
213. Vitale I, Sistigu A, Manic G, Rudqvist NP, Trajanoski Z, Galluzzi L. Mutational and antigenic landscape in tumor progression and cancer immunotherapy. Trends Cell Biol. 2019;29(5):396–416. doi:10.1016/j.tcb.2019.01.003.
214. Buchan SL, Fallatah M, Thirdborough SM, Taraban VY, Rogel A, Thomas LJ, Penfold CA, He L-Z, Curran MA, Keler T, et al. PD-1 blockade and CD27 stimulation activate distinct transcriptional programs that synergize for CD8+T-cell–driven antitumor

immunity. Clin Cancer Res. 2018;24(10):2383–2394. doi:10.1158/1078-0432.CCR-17-3057.
215. Aranda F, Vacchelli E, Eggermont A, Galon J, Fridman WH, Zitvogel L. Trial watch: immunostimulatory monoclonal antibodies in cancer therapy. Oncoimmunology. 2014;3(2):e27297. doi:10.4161/onci.27297.
216. Ansell SM. Pembrolizumab: living up to expectations. Blood. 2019;134(14):1114–1115. doi:10.1182/blood.2019002417.
217. Anandasabapathy N, Breton G, Hurley A, Caskey M, Trumpfheller C, Sarma P, Pring J, Pack M, Buckley N, Matei I, et al. Efficacy and safety of CDX-301, recombinant human Flt3L, at expanding dendritic cells and hematopoietic stem cells in healthy human volunteers. Bone Marrow Transplant. 2015;50(7):924–930. doi:10.1038/bmt.2015.74.
218. Satyamitra M, Cary L, Dunn D, Holmes-Hampton GP, Thomas LJ, Ghosh SP. CDX-301: a novel medical countermeasure for hematopoietic acute radiation syndrome in mice. Sci Rep. 2020;10(1):1757. doi:10.1038/s41598-020-58186-1.
219. Cohen EEW, Bell RB, Bifulco CB, Burtness B, Gillison ML, Harrington KJ. The society for immunotherapy of cancer consensus statement on immunotherapy for the treatment of squamous cell carcinoma of the head and neck (HNSCC). J Immunother Cancer. 2019;7(1):184. doi:10.1186/s40425-019-0662-5.
220. Emens LA. Breast cancer immunotherapy: facts and hopes. Clin Cancer Res. 2018;24(3):511–520. doi:10.1158/1078-0432.CCR-16-3001.
221. Beer TM, Kwon ED, Drake CG, Fizazi K, Logothetis C, Gravis G, Ganju V, Polikoff J, Saad F, Humanski P, et al. Randomized, double-blind, phase III trial of ipilimumab versus placebo in asymptomatic or minimally symptomatic patients with metastatic chemotherapy-naive castration-resistant prostate cancer. J Clin Oncol. 2017;35(1):40–47. doi:10.1200/JCO.2016.69.1584.
222. Di Lorenzo G, Buonerba C, Kantoff PW. Immunotherapy for the treatment of prostate cancer. Nat Rev Clin Oncol. 2011;8(9):551–561. doi:10.1038/nrclinonc.2011.72.
223. Drake CG. Prostate cancer as a model for tumour immunotherapy. Nat Rev Immunol. 2010;10(8):580–593. doi:10.1038/nri2817.
224. Kwon ED, Drake CG, Scher HI, Fizazi K, Bossi A, van den Eertwegh AJ, Krainer M, Houede N, Santos R, Mahammedi H, et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol. 2014;15(7):700–712. doi:10.1016/S1470-2045(14)70189-5.
225. Costantini A, Julie C, Dumenil C, Helias-Rodzewicz Z, Tisserand J, Dumoulin J, Giraud V, Labrune S, Chinet T, Emile J-F, et al. Predictive role of plasmatic biomarkers in advanced non-small cell lung cancer treated by nivolumab. Oncoimmunology. 2018;7:e1452581. doi:10.1080/2162402X.2018.1452581.
226. Lo SS, Fakiris AJ, Chang EL, Mayr NA, Wang JZ, Papiez L, Teh BS, McGarry RC, Cardenes HR, Timmerman RD, et al. Stereotactic body radiation therapy: a novel treatment modality. Nat Rev Clin Oncol. 2010;7(1):44–54. doi:10.1038/nrclinonc.2009.188.
227. Bentebibel SE, Hurwitz ME, Bernatchez C, Haymaker C, Hudgens CW, Kluger HM, Tetzlaff MT, Tagliaferri MA, Zalevsky J, Hoch U, et al. A first-in-human study and biomarker analysis of NKTR-214, a novel IL2Rbetagamma-biased cytokine, in patients with advanced or metastatic solid tumors. Cancer Discov. 2019;9(6):711–721. doi:10.1158/2159-8290.CD-18-1495.
228. Charych DH, Hoch U, Langowski JL, Lee SR, Addepalli MK, Kirk PB, Sheng D, Liu X, Sims PW, VanderVeen LA, et al. NKTR-214, an engineered cytokine with biased IL2 receptor binding, increased tumor exposure, and marked efficacy in mouse tumor models. Clin Cancer Res. 2016;22(3):680–690. doi:10.1158/1078-0432.CCR-15-1631.
229. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. Nat Rev Immunol. 2012;12(3):180–190. doi:10.1038/nri3156.
230. Makarov DV, Carter HB. The discovery of prostate specific antigen as a biomarker for the early detection of adenocarcinoma of the prostate. J Urol. 2006;176(6):2383–2385. doi:10.1016/j.juro.2006.08.019.
231. Chang SS. Overview of prostate-specific membrane antigen. Rev Urol. 2004;6:S13–8.
232. Shore ND, Morrow MP, McMullan T, Kraynyak KA, Sylvester A, Bhatt K, Cheung J, Boyer JD, Liu L, Sacchetta B, et al. CD8+ T cells impact rising PSA in biochemically relapsed cancer patients using immunotherapy targeting tumor-associated antigens. Mol Ther. 2020;28(5):1238–1250. doi:10.1016/j.ymthe.2020.02.018.
233. Aucouturier J, Dupuis L, Deville S, Ascarateil S, Ganne V. Montanide ISA 720 and 51: a new generation of water in oil emulsions as adjuvants for human vaccines. Expert Rev Vaccines. 2002;1(1):111–118. doi:10.1586/14760584.1.1.111.
234. Turaj AH, Hussain K, Cox KL, MJJ R-Z, Testa J, Dahal LN, Chan HTC, James S, Field VL, Carter MJ, et al. Antibody tumor targeting is enhanced by CD27 agonists through myeloid recruitment. Cancer Cell. 2017;32(6):777–91 e6. doi:10.1016/j.ccell.2017.11.001.
235. Burris HA, Infante JR, Ansell SM, Nemunaitis JJ, Weiss GR, Villalobos VM, Sikic BI, Taylor MH, Northfelt DW, Carson WE, et al. Safety and activity of varlilumab, a novel and first-in-class agonist anti-CD27 antibody, in patients with advanced solid tumors. J Clin Oncol. 2017;35(18):2028–2036. doi:10.1200/JCO.2016.70.1508.
236. Starzer AM, Berghoff AS. New emerging targets in cancer immunotherapy: CD27 (TNFRSF7). ESMO Open. 2020;4(Suppl 3):e000629. doi:10.1136/esmoopen-2019-000629.
237. Ciotti M, Angeletti S, Minieri M, Giovannetti M, Benvenuto D, Pascarella S, Sagnelli C, Bianchi M, Bernardini S, Ciccozzi M, et al. COVID-19 outbreak: an overview. Chemotherapy. pp.1–9. 2020. doi:10.1159/000507423
238. Raoult D, Zumla A, Locatelli F, Ippolito G, Kroemer G. Coronavirus infections: epidemiological, clinical and immunological features and hypotheses. Cell Stress. 2020;4(4):66–75. doi:10.15698/cst2020.04.216.
239. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020;109:102433. doi:10.1016/j.jaut.2020.102433.