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Trial Watch

Toll-like receptor agonists for cancer therapy

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Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BCG, bacillus Calmette-Guérin; CRT, calreticulin; CTCL, cutaneous T-cell lymphoma; CTLA4, cytotoxic T-lymphocyte-associated protein 4; DAMP, damage-associated molecular pattern; DC, dendritic cell; dsRNA, double-stranded RNA; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; ERBB2, *v-erb-b2* erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian); GM-CSF, granulocyte macrophage colony-stimulating factor; HMGB1, high mobility group box 1; HNSCC, head and neck squamous cell carcinoma; HSP, heat-shock protein; ICD, immunogenic cell death; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; MAMP, microbe-associated molecular pattern; MDS, myelodysplastic syndrome; MLANA, melan-A; MPL, monophosphoryl lipid A; mtDNA, mitochondrial DNA; MUC1, mucin 1; nBCC, nodular basal cell carcinoma; NK, natural killer; NMIBC, non muscle-invasive bladder carcinoma; NSCLC, non-small cell lung carcinoma; ODN, oligodeoxynucleotide; PI3K, phosphoinositide-3-kinase; polyI:C, polyriboinosinic polyribocytidylic acid; PRAME, preferentially expressed antigen in melanoma; ssRNA, single-stranded RNA; TAA, tumor-associated antigen; TLR, Toll-like receptor; Treg, regulatory T cell

Toll-like receptors (TLRs) have long been known for their ability to initiate innate immune responses upon exposure to conserved microbial components such as lipopolysaccharide (LPS) and double-stranded RNA. More recently, this family of pattern recognition receptors has been attributed a critical role in the elicitation of anticancer immune responses, raising interest in the development of immunochemotherapeutic regimens based on natural or synthetic TLR agonists. In spite of such an intense wave of preclinical and clinical investigation, only three TLR agonists are currently licensed by FDA for use in cancer patients: bacillus Calmette–Guérin (BCG), an attenuated strain of *Mycobacterium bovis* that operates as a mixed TLR2/TLR4 agonist; monophosphoryl lipid A (MPL), a derivative of *Salmonella minnesota* that functions as a potent agonist of TLR4; and imiquimod, a synthetic imidazoquinoline that activates TLR7. One year ago, in the August and September issues of *OncoImmunology*, we described the main biological features of TLRs and discussed the progress of clinical studies evaluating the safety and therapeutic potential of TLR agonists in cancer patients. Here, we summarize the latest developments in this exciting area of research, focusing on preclinical studies that have been published during the last 13 mo and clinical trials launched in the same period to investigate the antineoplastic activity of TLR agonists.

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Introduction

Although the *Toll* gene was originally identified as a controller of the dorsal-ventral embryonic polarity of *Drosophila melanogaster* as early as in 1985,[1,2] its critical role in the response of fruit flies to fungal infections became clear only 10 y later.[3] Approximately in the same period, human orthologs of Toll begun to be characterized[4,5] and implicated in innate immune responses to bacterial lipopolysaccharide (LPS).[6–8] Since then, the murine genome has been shown to encode 13 distinct Toll-like receptors (TLRs), 10 of which are also coded by the human genome, and members of the TLR family have been discovered in evolutionarily distant organisms such as plants and fish.[9–11]

TLRs are enzymatically-inactive single membrane-spanning proteins best known for their ability to detect so-called

"microbe-associated molecular patterns" (MAMPs), conserved microbial products including (but not limited to) bacterial LPS and derivatives thereof (which generally operate as mixed TLR2/TLR4 agonists),[12–14] components of the bacterial cell wall, such as lipoteichoic acid (a specific activator of TLR2),[15] bacterial flagellin (a pure TLR5 agonist),[16–19] microbial DNA (mostly functioning as a TLR9 agonist),[20] microbial single-stranded RNA (ssRNA, which can be detected by both TLR7 and TLR8)[21–23] and viral double-stranded RNA (dsRNA, which specifically activates TLR3).[24–26] Of note, TLRs that detect nucleic acids (i.e., TLR3, TLR7, TLR8 and TLR9) are localized to the endosomal compartment, while TLRs that mainly detect proteolipidic structures (i.e., TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10) are exposed on the cell surface.[27,28] As an exception to this general pattern, TLR2 and TLR10 (the sole orphan TLR in humans) have been shown to co-localize at phagosomes, perhaps indicating that TLR10 shares some binding specificity with TLR2. Compelling evidence in support of this hypothesis, however, is missing. Along similar lines, the actual role of murine Tlr11, Tlr12 and Tlr13 has just begun to emerge (see below).

Several TLRs have recently been shown to sense not only exogenous MAMPs but also endogenous "damage-associated molecular patterns" (DAMPs), i.e., molecules released or exposed by stressed, dying or dead cells to convey a danger signal.[29–32] These DAMPs include, but presumably are not limited to: several heat-shock proteins (e.g., HSP60, HSP70),[33,34] uric acid[35] and surfactant protein A,[36] all of which function as mixed TLR2/TLR4 agonists; the non-histone chromatin-binding protein high mobility group box 1 (HMGB1) and the Ca^{2+}- and Zn^{2+}-binding protein S100A9, both operating as TLR4 agonists;[37–41] multiple components and breakdown products of the extracellular matrix, which mainly activate TLR4;[42] and mitochondrial DNA (mtDNA), a pure TLR9 agonist.[43,44] Interestingly nuclear DNA from eukaryotic cells can also be recognized by TLR9 if the latter is ectopically expressed at the plasma membrane (rather than in endosomes).[45] This suggests that TLR9 might specifically respond to exogenous (as opposed to self) DNA because of its own subcellular localization rather than due to the methylation state and frequency of CpG islands on its ligand (as originally thought).[20,46] A detailed description of the signaling cascades triggered by TLRs in response to MAMPs or DAMPs exceeds the scope of the present Trial Watch and can be found in refs. 27 and 47–50.

The spatiotemporally defined emission of specific DAMPs by dying cells has been proposed to constitute the essence of immunogenic cell death (ICD), a peculiar type of apoptosis that activates adaptive immune responses.[39,51,52] So far, only a few bona fide inducers of ICD have been identified: specific chemotherapeutic agents such as mitoxantrone, doxorubicin and oxaliplatin, ionizing irradiation ad some types of photodynamic therapy. DAMPs that play a prominent role in ICD include (but presumably are not limited to) the endoplasmic reticulum (ER) chaperone calreticulin (CRT), ATP, HSP70 and HMGB1.[39,52,53] Importantly, both HSP70 and HMGB1 appear to exert immunostimulatory functions by activating TLR4 on the surface of antigen-presenting cells, hence promoting the cross-priming of antigen-specific T lymphocytes.[54–56] Thus, TLRs appear to play a prominent role not only in the orchestration of innate immune responses against infectious pathogens, but also in anticancer immunity, be it spontaneous or elicited by (chemo)therapeutic interventions.[23,27,57,58] In accord with this notion, functionally relevant polymorphisms in the genes encoding several TLRs (i.e., TLR1, TLR2, TLR3, TLR4, TLR6, TLR9 and TLR10) have been shown to influence the natural development of a wide array of neoplasms, including tumors that are not associated with a microbial etiology,[59–77] as well as to affect the response of cancer patients to chemotherapy and immunotherapy, at least in some settings.[54,78–80] Moreover, the expression of several TLRs including TLR2, TLR4, TLR7 and TLR9 by malignant cells appear to evolve not only along with oncogenesis and tumor progression, but also in response to microenvironmental cues,[81–90] suggesting that, at least in some types of cancer, TLRs may influence disease progression in a direct fashion rather than as a consequence of immunological effects.

Irrespective of the great preclinical and interest orbiting around TLRs since the late 1990s, however, only three TLR agonists are nowadays approved by FDA for use in cancer patients: bacillus Calmette–Guérin (BCG, an attenuated strain of *Mycobacterium bovis* initially developed as a vaccine against tuberculosis), which is currently approved for the immunotherapy of in situ bladder carcinoma;[57,91] monophosphoryl lipid A (MPL), a derivative of the LPS of *Salmonella minnesota*, which is currently licensed as part of Cervarix®, a vaccine against human papillomavirus 16 and 18 (the etiological determinants of > 70% cases of cervical carcinoma);[92,93] and imiquimod (a small imidazoquinoline derivative originally developed as a topic antiviral agent), which is currently used (as a 5% cream) for the treatment of actinic keratosis, superficial basal cell carcinoma and external genital or perianal warts (*condylomata acuminata*).[57] Of note, while both BCG and MPL function as mixed TLR2/TLR4 agonists,[14,94,95] imiquimod mainly exerts immunostimulatory effects in a TLR7-dependent manner.[96,97] Interestingly, also the Coley toxin, a mixture of killed *Streptococcus pyogenes* and *Serratia marcescens*,[98] is thought to mediate therapeutic effects by activating TLR2 and/or TLR4.[99–101] Nonetheless, the use of the Coley toxin as an anticancer medication has been discontinued in the 1960s, mostly due to concerns raised by the thalidomide case.[102]

One year ago, in the August and September issues of *OncoImmunology*, we presented the main functions of human TLRs in innate and cognate immunity and discussed the progress of recent clinical studies evaluating the safety and immunostimulatory activity of TLR agonists in cancer patients.[50,103] Here, along the lines of our monthly Trial Watch series,[50,103–117] we review the latest developments in this area of research, focusing on preclinical studies that have been published during the last 13 mo and clinical trials initiated in the same period to assess the antineoplastic potential of hitherto experimental TLR activators as well as of FDA-approved TLR agonists employed as "off-label" medications against cancer.

Literature Update

Clinical studies. During the last 13 mo, the results of no less than 28 clinical trials investigating the immunostimulatory potential

of TLR agonists (all confounded) in cancer patients have been published. Seventeen of these studies involved FDA-approved agents (i.e., BCG, MPL and imiquimod), most often employed as "on-label" medications, while the other 11 trials assessed the safety and therapeutic profile of hitherto experimental TLR agonists (source www.clinicaltrials.gov). Interestingly, none of the clinical studies published since May 1, 2012, have investigated the immunostimulatory potential of BCG in off-label oncological settings. Rather, the intravesical instillation BCG was chosen as a reference approach for the treatment of non muscle-invasive bladder carcinoma (NMIBC), and hence given to the control arm of the cohort,[118,119] or alternative dosing schedules were investigated.[120,121] Thus, the perioperative instillation of mitomycin C (a DNA crosslinker) or gemcitabine (a nucleoside analog) appears not to improve the therapeutic profile of intravesical BCG given as a standalone intervention.[118,119] Moreover, it has been shown that (1) a 2-week intravesical BCG maintenance regimen is virtually as efficient as a standard 3-week course;[120] (2) BCG administered at 1/3 of the standard dose causes the same side effects than the full-dose regimen among intermediate- and high-risk NMIBC patients;[121] and (3) individuals affected by high-risk (but not intermediate-risk) NMIBC benefit from a 3-year full-dose BCG maintenance therapy only in terms of tumor recurrence, but not of disease progression and overall survival.[121] Finally, the presence of cytogenetically abnormal cells as detected by fluorescence in situ hybridization has been proposed as a means of predicting the response of NMIBC patients to standard-of-care BCG immunotherapy.[122]

A single study of those mentioned above dealt with Cervarix®, which contains the MPL-based adjuvant AS04 (MPL + aluminum salts). This work actually demonstrated the immunogenicity and safety of Cervarix® co-administered with Twinrix®, an FDA-approved vaccine consisting in inactivated hepatitis A and B viral particles, in girls aged 9–15 y.[123] In addition, the hitherto experimental formulation AS15, that is, MPL + QS21 (a water soluble saponin extracted from the South American tree *Quillaja saponaria* Molina)[124] + CpG oligodeoxynucleotides (ODNs), has been employed to boost the immunogenicity of a *v-erb-b2* erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) (ERBB2)-targeting vaccine in patients with trastuzumab-resistant ERBB2-overexpressing metastatic breast carcinoma.[125] In this Phase I clinical trial, the co-administration of ERBB2-derived peptides with lapatinib, a relatively unspecific inhibitor of ERBB2 tyrosine kinase activity,[126] was well tolerated, triggered detectable ERBB2-specific immune responses in a majority of patients, and was associated with promising clinical benefits, warranting the initiation of Phase II/III studies.[125]

Imiquimod (also known as R-837) still stands out as the TLR agonist that generates the broadest clinical interest of all, presumably owing not only to its approval status, but also to the increasing amount of data from clinical studies and pharmacosurveillance demonstrating that—at least in its 5% cream formulation—imiquimod has an exceptional safety profile.[97,103] In line with this notion, no less than 12 studies investigating the immunostimulatory potential of imiquimod in cancer patients have been published during the last 13 mo (8 of which employing imiquimod as an off-label medication). Conversely, no reports on the immunostimulatory activity of resiquimod (R-848), another synthetic imidazoquinoline that operates as a TLR7 agonist, in cancer patients have been made in the same period, though the clinical interest in this compounds remains high (see below).

The combination of imiquimod 5% cream (commercialized by 3M Pharmaceuticals under the label of Aldara®) with aminolevulinate-based photodynamic therapy has been shown to provide therapeutic benefits to patients affected by actinic keratosis and basal cell carcinoma over the use of either intervention alone.[127,128] In addition, imiquimod 5% cream has been evaluated as a means to reduce the size of nodular basal cell carcinomas of the face, and hence limit the aesthetic impact of subsequent Mohs micrographic surgery, with encouraging results.[129] Besides being tested in such on-label indications, imiquimod has been investigated for its ability to exert immunostimulatory effects as an off-label medication, for instance (1) in melanoma patients receiving imiquimod as a standalone intervention or as an adjuvant to a nanoparticle-based vaccine;[130,131] (2) in subjects with cutaneous metastases of breast carcinoma or melanoma, receiving imiquimod alone or in combination with 5-fluorouracil (a nucleoside analog), respectively;[132,133] (3) in prostate and renal cancer patients, receiving imiquimod as an adjuvant to a dual peptide-based vaccine;[134] and (4) in women with high-grade cervical intraepithelial neoplasms and in HIV-1-infected men bearing anal intraepithelial tumors, two settings in which imiquimod was employed as a standalone therapeutic intervention.[135,136] Moreover, the safety and immunostimulatory potential of the intravesical instillation of a liquid formulation of imiquimod (TMX-101) have been assessed in a cohort of NMIBC patients.[137] Although imiquimod appeared to be inferior to other therapeutic modalities (notably electrocautery) in the treatment of HIV1-associated anal intraepithelial neoplasms,[136] these studies confirmed the safety of imiquimod and its ability to stimulate (natural or vaccine-induced) immune responses that—at least in a fraction of patients—engender clinical benefits.

Picibanil (OK-432) is a lyophilized preparation of *Streptococcus pyogenes* (operating as a mixed TLR2/TLR4 agonist) that has been approved for use in cancer patients by the Japanese Ministry of Health and Welfare as early as in 1975.[42,138] In line with this notion, the immunostimulatory activity of picibanil is being intensively investigated in Japan, but very much less so in the US and Europe. During the last 13 mo, picibanil has been shown to be well tolerated and effective as an adjuvant (1) to immature DCs administered intratumorally to resectable pancreatic cancer patients;[139] (2) to cisplatin (a DNA-damaging agent frequently associated with chemoresistance)[140] and hyperthermotherapy, in a cohort of individuals with malignant pleural effusions;[141] and (3) to a NY-ESO-1-based vaccine, in a single lung cancer patient selected out of a previously completed clinical trial[142] for the study of multiple immunological parameters.[143] In the same period, additional TLR2/TLR4 agonists have been shown to be safe and to exert therapeutically relevant immunostimulatory effects in cancer patients, including OM-174 (also known as CRX-527), a water soluble, diphosphorylated and triacetylated

form of lipid A from *Escherichia coli*,[144] which has been tested in patients with refractory solid tumors;[145] and IMM-101, a preparation of heat-killed *Mycobacterium obuense*[146] that has been investigated as a standalone therapeutic intervention against melanoma.[147] Moreover, recently completed trials have evaluated the safety and immunostimulatory profile of (1) the TLR3 agonist Hiltonol, a particular formulation of polyriboinosinic polyribocytidylic acid (polyI:C) that includes carboxymethylcellulose and poly-*L*-lysine as stabilizing agents,[148] employed as adjuvant of a peptide-derived vaccine against ovarian cancer;[149] (2) the imidazoquinoline 852A, a TLR7 agonist, administered subcutaneously for a prolonged period to patients with advanced hematologic malignancies;[150] (3) the TLR9 agonist IMO-2055 (an immunomodulatory oligonucleotide also known as EMD1201081),[151,152] given in combination with 5-fluorouracil, cisplatin, and cetuximab (an FDA-approved monoclonal antibody specific for the epidermal growth factor receptor, EGFR)[153] as a first-line palliative treatment to patients with recurrent/metastatic head and neck squamous cell carcinoma (HNSCC);[154] and (4) Agatolimod (also known as CpG-7909, PF-3512676 and Promune®), an unmethylated CpG ODN that also activates TLR9,[155] given in combination with tremelimumab (an experimental monoclonal antibody targeting the immune checkpoint regulator cytotoxic T-lymphocyte-associated protein 4, CTLA4)[156,157] to patients affected by advanced solid tumors (including melanoma),[158] or combined with local irradiation in subjects bearing mycosis fungoides.[159]

Preclinical studies. During the last 13 mo, TLR agonists have been the subject of an intense wave of preclinical investigation, resulting in more than 200 hundreds scientific publications (source www.ncbi.nlm.nih.gov/pubmed/). A large fraction of these studies has confirmed the ability of multiple TLR agonists to mediate—alone or combined with radio-, chemo- or immunotherapy—prophylactic or therapeutic effects against a variety of tumors, including (but not limited to) lymphoma,[160–162] mastocytoma,[162] glioma,[163] breast carcinoma,[164,165] thymoma,[166] fibrosarcoma,[167] head and neck cancer,[168,169] melanoma,[170–174] lung cancer,[174–176] colorectal carcinoma,[174,177–179] renal cancer[177,180] and ovarian carcinoma.[181,182] For the most part, such robust antineoplastic effects have been ascribed to the ability of TLR agonists to induce de novo or boost pre-existing (natural or therapy-elicited) immune responses. This said, accumulating preclinical evidence suggests that TLR-targeting agents can also influence tumor progression in a direct manner as they interact with TLRs expressed on the surface of malignant cells. Thus, activators of TLR3 (e.g., dsRNA, polyI:C),[183–186] TLR4 (e.g., picibanil, LPS)[187,188] and TLR7 (e.g., imiquimod)[189] have all been shown to arrest the proliferation or induce the death of various cancer cell lines in vitro, in the absence of immune effectors. However, the direct effects of TLR agonists on malignant cells exhibit a consistent degree of context-dependency. In line with this notion, TLR-conveyed signals have also been shown to confer malignant cells with a proliferative advantage,[190–201] with an increased invasive/metastatic potential,[193,194,196–200,202–208] with a profound resistance to environmental and chemotherapeutic cues,[192,195,209–212] or with the ability to secrete immunosuppressive cytokines.[201,211,213] Of note, endothelial cells have been shown to respond to the release of peroxiredoxin 1 (a TLR4-interacting DAMP) from dying prostate cancer cells by secreting vascular endothelial growth factor, thus stimulating angiogenesis.[214] Thus, TLR agonists may promote tumor progression not only as they directly alter the behavior of malignant cells, but also as they engage signaling circuitries that involve the tumor stroma. Taken together, these observations suggest that the therapeutic potential of a given TLR agonist in a given clinical setting should be carefully evaluated in view of its (more or less pronounced) propensity to mediate direct or indirect pro-tumor effects.

The discovery/development of novel TLR agonists is also a very active area of research and during the past 13 mo several new compounds and strategies to activate specific TLRs have been reported. Great interest has gathered around the use of cationic preparations, notably liposomes, as a means to deliver immunotherapeutic agents (e.g., TLR agonists, vaccines) to neoplastic lesions.[215–217] Indeed, cationic agents may per se exert immunostimulatory effects by binding to, and hence activating TLR4.[216] In addition, several new TLR agonists have been identified, including endogenous DAMPs as well as exogenous chemicals. Thus, heparan sulfate, a component of the extracellular matrix, has turned out to bind TLR4 on the surface of DCs, hence promoting their maturation (in vitro), and to be involved in the etiology of the graft- vs. -host disease.[218] Along similar lines, multiple mature microRNAs have been demonstrated to activate natural killer (NK) cells, in vitro and in vivo, in a TLR1-dependent manner, hence protecting mice from a challenge with A20 lymphoma cells,[219] while two specific microRNAs secreted by tumor cells, namely, miR-21 and miR-29a, have been reported to mediate pro-metastatic effects by stimulating a TLR7-dependent inflammatory response.[220] A novel, chemically-defined LPS derivative has been reported to reinstate the immunogenicity of HGMB1-deficient cancer cells, thus compensating for cell-intrinsic alterations that may compromise the therapeutic efficacy of ICD inducers.[221] Finally, extracts of *Larix kaempferi* (a popular Japanese larch) have been shown to exert promising immunostimulatory effects in a murine model of thymoma as they activate TLR2 and TLR4,[222] while distinct isothiocyanates have been reported to differentially modulate (either promote or inhibit) TLR3-dependent signal transduction cascades.[223]

Several papers published since May 1, 2012, have investigated the fundamental mechanisms whereby TLRs exert a prominent influence not only on immune effector cells but also on malignant and stromal cells. Among dozens of top quality reports, we have found the following works of particular interest. Shi et al. have identified a role for Tlr11 in the control of *Salmonella spp* infection at the level of Peyer patches,[224] while Oldenburg and colleagues have discovered (one of) the natural ligand(s) of Tlr13, namely, bacterial 23S rRNA.[225] Several groups have demonstrated that specific microRNAs play a critical function in the signaling cascades elicited by various TLRs, including TLR4 and TLR9, in both immune and malignant cells.[198,226–228] Dibra et al. have demonstrated that TLR9 signaling in macrophages and CD3 signaling in T cells underpin a cellular crosstalk that result in the secretion of high levels of interleukin (IL)-30.[229] Balamurugan

and colleagues have unveiled a molecular cascade linking the oncosuppressive activity of FBXW7 to the transcriptional repression of *TLR4*,[230] whereas Aksoy et al. have reported that the subcellular compartmentalization of TLR4 is under the control of phosphoinositide-3-kinase (PI3K).[231] As PI3K is frequently hyperactivated in malignant cells,[232] these findings lend further support to the notion that TLR4 may be implicated in tumor-cell intrinsic oncogenic signaling cascades.[79,188,211,233] By generating induced pluripotent stem cells from the dermal fibroblasts of TLR3-deficient patients and healthy individuals, Lafaille and coworkers have implicated TLR3 in the cell-intrinsic immunity of neurons and oligodendrocytes to herpes simplex virus 1.[234] The research group lead by Richard Gallo has demonstrated that UV rays induce alterations in the double-stranded domains of some non-coding RNAs, endowing them with the ability to operate as DAMPs and activate TLR3.[235] Luger and colleagues have shown that TLR4-activated DCs switch from an initial pro-inflammatory mode, characterized by the release of T_H1 cytokines including IL-12 and interferon (IFN)γ, to an anti-inflammatory one, featuring the autocrine secretion of IL-10.[236] During this latter functional phase, DCs reportedly become able to inhibit the proliferation of T cells and to convert them into IL-10, FOXP3-expressing regulatory T cells (Tregs).[236–239] Karbach and coworkers have demonstrated that the use of Agatolimod is associated with the formation of neutralizing antibodies in significant fraction of patients, de facto abolishing its long-term therapeutic potential.[240] According to Lin et al., TLR2 is required for the removal of senescent hepatocytes by immune effector cells, hence restraining the development of hepatocellular carcinoma.[241,242] Ochi et al. have implicated TLR4 and TLR7 in pancreatic carcinogenesis, in both mice and humans, presumably linked to their robust pro-inflammatory functions.[243–245] Hodven and collaborators have reported that picibanil not only activates TLR2 and TLR4, but also exert TLR3-dependent immunostimulatory effects.[246] Finally, Walter et al. have discovered that Aldara® stimulates inflammatory responses also in an imiquimod- and TLR7-independent manner.[247] Indeed, an abundant component of Aldara®, isostearic acid, appears to trigger the activation of the inflammasome, hence stimulating the release of IL-1β and IL-18,[248] even in the absence of imiquimod. Altogether, these findings suggest (1) that the biological functions of TLRs are complex and exhibit a significant degree of context-dependency, and (2) that the signal transduction cascades originating from TLRs are intimately intertwined with several other cell-intrinsic and cell-extrinsic signaling pathways. Much work is still needed to characterize this functional crosstalk, which has profound implications for cancer (immuno)therapy.

Update on Clinical Trials

When this Trial Watch was being redacted (May 2013), official sources listed only 32 clinical trials launched after May 1, 2012, to investigate the safety and therapeutic potential of experimental and FDA-approved TLR agonists (source www.clinicaltrials.gov). Of these, 7 involved BCG (1 study), AS04 (3 studies) or imiquimod (3 studies) as fully "on-label" medications, and therefore will not be discussed further here. Of the remaining 25 clinical trials, 10 aimed at assessing the immunostimulatory potential of BCG or imiquimod in "off-label" oncological settings, and 15 were launched to test the safety and antineoplastic activity of hitherto investigational TLR agonists (**Table 1**).

Recently initiated clinical trials are testing BCG, either in combination with the FDA-approved immunostimulatory monoclonal antibody ipilimumab[78,249–251] (NCT01838200) or as an adjuvant to irradiated allogeneic melanoma cells plus recombinant granulocyte macrophage colony-stimulating factor (GM-CSF, Molgramostin®) (NCT01729663), only in advanced melanoma patients. Ad odds with such an apparent decrease in the clinical interest generated by BCG, the possibility to use of imiquimod as an immunostimulatory agent in patients affected by a variety of tumors remains under intensive investigation. In particular, during the last 13 mo clinical trials have been initiated that aim at assessing the safety and therapeutic efficacy of imiquimod (1) employed as a standalone intervention to minimize the risk of recurrence as well as the esthetic impact of surgery in patients bearing lentigo maligna melanomas on the head and face (NCT01720407); (2) given in support of DCs loaded with tumor cell lysates to pediatric and adult sarcoma patients who have optionally been pre-conditioned with gemcitabine (to inhibit myeloid-derived suppressor cells)[252–255] (NCT01803152); (3) used as a topic support to a DC-based vaccine in adult and pediatric patients affected by anaplastic astrocytoma, glioma or glioblastoma multiforme (NCT01808820), or to a tumor cell lysate-based vaccine in individuals with high-grade/recurrent glioma[256] (NCT01678352); (4) given as an adjuvant to HLA-A2-restricted tumor-associated antigen (TAA)-derived peptides in children bearing recurrent ependymomas (NCT01795313); (5) used as a standalone intervention in the form of anal suppositories (as an alternative to ablative therapy) to men who had sex with HIV-1-infected men (NCT01663558); or (6) employed as a primary measure in alternative to surgery in women affected by vulvar intraepithelial neoplasia (NCT01861535). Moreover, following the encouraging results of a recent Phase I clinical trial,[137] a Phase II study has been initiated to test the therapeutic activity of a liquid imiquimod preparation (TMX-101) administered intravesically to NMIBC patients (NCT01731652).

Also the general interest in experimental TLR agonists appears to decrease, with the notable exception of Hiltonol™, a particular formulation of polyriboinosinic polyribocytidylic acid (polyI:C, Ampligen™, Rintatolimod) that includes carboxymethylcellulose and poly-l-lysine as stabilizing agents.[148] Thus, the safety and immunostimulatory potential of Hiltonol™, which operates as a TLR3 agonist, are being investigated in (1) acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) patients receiving a recombinant vaccine consisting of full-length NY-ESO-1 fused to an anti-LY75 monoclonal antibody in combination with 5-aza-2'-deoxycytidine (an FDA-approved analog of cytidine also known as decitabine)[257] (NCT01834248); (2) multiple myeloma (MM) patients treated with a multipeptide vaccine (PVX-410) (NCT01718899); (3) melanoma patients administered with a NY-ESO-1 based vaccine plus ipilimumab (NCT01810016); (4) unresectable pancreatic carcinoma

Table 1. Recent clinical trials evaluating Toll-like receptor agonists in cancer patients*

Agent	Target	Cancer type	Status	Phase	Route	Notes	Ref.
AS15	TLR2 TLR4 TLR9	NSCLC	Not yet recruiting	II	i.m.	As single agent	NCT01853878
BCG	TLR2 TLR4	Melanoma	Recruiting	I	i.t.	Combined with ipilimumab	NCT01838200
				II/III	n.a.	Combined with irradiated melanoma cells and rhGM-CSF	NCT01729663
CBLB502	TLR5	HNSCC	Not yet recruiting	I	s.c.	Combined with cisplatin and IMRT	NCT01728480
GNKG168	TLR9	ALL AML	Active, not recruiting	I	i.v.	As single agent	NCT01743807
Hiltonol™	TLR3	AML MDS	Recruiting	I	s.c.	Combined with recombinant vaccine and decitabine	NCT01834248
		Melanoma	Recruiting	I	s.c.	Combined with a NY-ESO-1-based vaccine and ipilimumab	NCT01810016
		MM	Active, not recruiting	I	i.m.	Combined with a multi-peptide vaccine (PVX-410)	NCT01718899
		NSCLC	Recruiting	I/II	s.c.	Combined with MUC1-targeting vaccination	NCT01720836
		Pancreatic cancer	Recruiting	0	i.t.	Combined with immature DCs	NCT01677962
		Solid tumors	Active, not recruiting	II	n.a.	Combined with autologous DCs	NCT01734564
Imiquimod	TLR7	Anal dysplasia	Not yet recruiting	IV	Local	As single agent	NCT01663558
		Astrocytoma GBM Glioma	Not yet recruiting	I	Topical	Combined with DC-based vaccination	NCT01808820
		Ependymoma	Recruiting	n.a.	Topical	Combined with HLA-A2-restricted TAA-derived peptides	NCT01795313
		Glioma	Recruiting	0	Topical	Combined with a tumor cell lysate-based vaccine	NCT01678352
		Lentigo maligna melanoma	Recruiting	III	Topical	As a standalone neoadjuvant intervention	NCT01720407
		Sarcoma	Recruiting	I	Topical	Combined with DC-based vaccination ± gemcitabine	NCT01803152
		VIE	Not yet recruiting	III	Topical	As an alternative to primary surgery	NCT01861535
Resiquimod	TLR7 TLR8	CTCL	Recruiting	I/II	Topical	As single agent	NCT01676831
		Melanoma	Recruiting	n.a.	Topical	Combined with MLANA-targeting vaccination	NCT01748747
		nBCC	Recruiting	I/II	Topical	As single agent	NCT01808950
SD-101	TLR9	Lymphoma	Recruiting	I	i.t.	Combined with radiotherapy	NCT01745354
TMX-101	TLR7	NMIBC	Recruiting	II	Intravesical	As single agent	NCT01731652
VTX-2337	TLR8	HNSCC	Not yet recruiting	II	n.a.	Combined with conventional chemotherapy plus cetuximab	NCT01836029
		Reproductive tract cancer	Recruiting	II	n.a.	Combined with PLD	NCT01666444

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BCG, bacillus Calmette-Guérin; CTCL, cutaneous T-cell lymphoma; DC, dendritic cell; GBM, glioblastoma; HNSCC, head and neck squamous cell carcinoma; i.m., intra musculum; IMRT, intensity-modulated radiation therapy; i.t., intra tumorem; i.v., intra venam; MLANA, melan-A; MDS, myelodysplastic syndrome; MM, multiple myeloma; MUC1, mucin 1; n.a., not available; nBCC, nodular basal cell carcinoma; NMIBC, non-muscle-invasive bladder carcinoma; NSCLC, non-small cell lung carcinoma; PLD, pegylated liposomal doxorubicin; rhGM-CSF, recombinant human granulocyte-macrophage colony-stimulating factor; s.c., sub cutem; TAA, tumor-associated antigen; TLR, Toll-like receptor; VIE, vulvar intraepithelial neoplasia. *Started after May 1, 2012.

patients receiving immature DCs[258] (NCT01677962); (5) individuals administered with a mucin 1 (MUC1)-derived peptide vaccine for the treatment of non-small cell lung carcinoma (NSCLC) (NCT01720836); and (6) subjects bearing not better specified neoplasms and allocated to receive autologous DCs (NCT01734564).

The TLR5 agonist CBLB502 (a derivative of Salmonella flagellin also known as Entolimod) is being tested in Stage III–IV HNSCC patients receiving cisplatin and radiation therapy (NCT01728480), reflecting the robust immunostimulatory and radioprotective activities that have been ascribed to this compound in preclinical settings.[259–262] The safety and immunostimulatory profile of the TLR7 agonist resiquimod are being assessed (1) in cohorts of cutaneous T-cell lymphoma (CTCL) and nodular basal cell carcinoma (nBCC) patients, two settings in which resiquimod is administered topically as a standalone therapeutic intervention (NCT01676831; NCT01808950); as well as (2) in patients with recurrent or advanced melanoma, receiving resiquimod or an HIV-1-derived peptide as alternative adjuvants to a melan-A (MLANA)-targeting vaccine (NCT01748747). VTX-2337, a small molecule that specifically binds and activates TLR8,[168] is under investigation for its ability to improve the therapeutic response of HNSCC patients to conventional chemotherapy plus cetuximab (NCT01836029) and that of women bearing reproductive tract neoplasms to pegylated liposomal doxorubicin (NCT01666444). Finally, a few clinical trials have recently been initiated to evaluate the immunostimulatory potential of TLR9 agonists in cancer patients. In particular, (1) AS15 (which actually operates as a mixed TLR4/TLR9 agonist, as it contains both MPL and Agatolimod) is being tested for its ability to boost immune responses elicited by full-length recombinant preferentially expressed antigen in melanoma (PRAME) in NSCLC patients upon tumor resection (NCT01853878); (2) the safety and therapeutic profile of GNKG168 (a CpG ODN),[263,264] given intravenously as a standalone agent, are being assessed in a cohort of relapsed acute lymphoblastic leukemia (ALL) or AML patients (NCT01743807); and (3) the intratumoral administration of SD-101 (a phosphorothiolate CpG ODN)[263] is being evaluated as a means to exacerbate the antineoplastic effects of local irradiation in subjects bearing Hodgkin and non-Hodgkin lymphoma (NCT01745354).

Of note, during the last 13 mo, 11 clinical trials investigating the immunostimulatory activity of TLR agonists in cancer patients that had been registered at www.clinicaltrials.gov after January 1, 2008, are now listed as terminated (2 studies), suspended (1 study) or completed (9 studies). In particular, NCT01013623 (testing BCG as a standalone agent in melanoma patients) and NCT01396018 (assessing the activity of VTX-2337 in combination with radiotherapy in B-cell lymphoma patients) have been terminated owing to a lack of accrual and to an excessively slow rate of recruitment, respectively, while NCT01400672 (investigating the immunostimulatory potential of a tumor cell lysate-based vaccine adjuvanted with imiquimod in individuals bearing glioma or glioblastoma) has been suspended for safety concerns. The results of only 1 (NCT00707174) of the 9 studies that are nowadays listed as completed (NCT00006352; NCT00079300; NCT00581425; NCT00707174; NCT00773097; NCT00785122; NCT00952692; NCT01161888; NCT01219348) have been published, indicating that the administration of topical retinoid may reduce the risk of local recurrence among lentigo maligna melanoma patients treated with imiquimod 5% cream.[265]

Concluding Remarks

There is an abundant literature demonstrating that TLR agonists can exert potent immunostimulatory effects in vivo, hence triggering de novo or boosting pre-existing (natural or therapy-elicited) anticancer immune responses.[23,27,47,52,58,266] Nonetheless, only a few TLR agonists are nowadays licensed by international regulatory agencies for use in cancer patients, i.e., BCG, MPL, imiquimod (all of which are approved by the US FDA) and picibanil (which is approved by the Japanese Ministry of Health and Welfare). In addition, the number or clinical trials that are initiated to test the safety and therapeutic profile of TLR agonists in oncological indications is steadily decreasing. Thus, the trend that we have delineated one year ago, in the August and September issues of *OncoImmunology*,[50,103] appears to be confirmed. Such a decline has surely been influenced by limited availability of clinical grade TLR agonists, prompting academic researchers to focus on surrogate compounds.[267,268] Moreover, most (if not all) TLR agonists activate a complex set of signal transduction cascades that involve not only immune effectors but also malignant cells and components of the tumor stroma. As the biological outcomes of such a cell-intrinsic and cell-extrinsic signaling network exhibit an elevated degree of context-dependency, it is possible—yet remains to be formally demonstrated—that TLR agonists induce therapeutic responses only in specific patient subsets. Thus, the future of these immunostimulatory agents might depend not only on the precise elucidation of the signaling pathways that they activate at the cell-intrinsic and cell-extrinsic level, but also on the identification of biological markers that predict the propensity of individual cancer patients to obtain a clinical benefit from TLR agonists.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Anderson KV, Bokla L, Nüsslein-Volhard C. Establishment of dorsal-ventral polarity in the Drosophila embryo: the induction of polarity by the Toll gene product. Cell 1985; 42:791-8; PMID:3931919; http://dx.doi.org/10.1016/0092-8674(85)90275-2
2. Anderson KV, Jürgens G, Nüsslein-Volhard C. Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role of the Toll gene product. Cell 1985; 42:779-89; PMID:3931918; http://dx.doi.org/10.1016/0092-8674(85)90274-0
3. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 1996; 86:973-83; PMID:8808632; http://dx.doi.org/10.1016/S0092-8674(00)80172-5
4. Nomura N, Miyajima N, Sazuka T, Tanaka A, Kawarabayasi Y, Sato S, et al. Prediction of the coding sequences of unidentified human genes. I. The coding sequences of 40 new genes (KIAA0001-KIAA0040) deduced by analysis of randomly sampled cDNA clones from human immature myeloid cell line KG-1. DNA Res 1994; 1:27-35; PMID:7584026; http://dx.doi.org/10.1093/dnares/1.1.27
5. Taguchi T, Mitcham JL, Dower SK, Sims JE, Testa JR. Chromosomal localization of TIL, a gene encoding a protein related to the Drosophila transmembrane receptor Toll, to human chromosome 4p14. Genomics 1996; 32:486-8; PMID:8838819; http://dx.doi.org/10.1006/geno.1996.0150
6. Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 1997; 388:394-7; PMID:9237759; http://dx.doi.org/10.1038/41131
7. Yang RB, Mark MR, Gray A, Huang A, Xie MH, Zhang M, et al. Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. Nature 1998; 395:284-8; PMID:9751057; http://dx.doi.org/10.1038/26239
8. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 1998; 282:2085-8; PMID:9851930; http://dx.doi.org/10.1126/science.282.5396.2085
9. Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, et al. A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. Science 1995; 270:1804-6; PMID:8525370; http://dx.doi.org/10.1126/science.270.5243.1804
10. Gómez-Gómez L, Boller T. FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol Cell 2000; 5:1003-11; PMID:10911994; http://dx.doi.org/10.1016/S1097-2765(00)80265-8
11. Roach JC, Glusman G, Rowen L, Kaur A, Purcell MK, Smith KD, et al. The evolution of vertebrate Toll-like receptors. Proc Natl Acad Sci U S A 2005; 102:9577-82; PMID:15976025; http://dx.doi.org/10.1073/pnas.0502272102
12. Jiang Q, Akashi S, Miyake K, Petty HR. Lipopolysaccharide induces physical proximity between CD14 and toll-like receptor 4 (TLR4) prior to nuclear translocation of NF-kappa B. J Immunol 2000; 165:3541-4; PMID:11034352
13. Schröder NW, Opitz B, Lamping N, Michelsen KS, Zähringer U, Göbel UB, et al. Involvement of lipopolysaccharide binding protein, CD14, and Toll-like receptors in the initiation of innate immune responses by Treponema glycolipids. J Immunol 2000; 165:2683-93; PMID:10946299
14. Mata-Haro V, Cekic C, Martin M, Chilton PM, Casella CR, Mitchell TC. The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. Science 2007; 316:1628-32; PMID:17569868; http://dx.doi.org/10.1126/science.1138963
15. Hoebe K, Georgel P, Rutschmann S, Du X, Mudd S, Crozat K, et al. CD36 is a sensor of diacylglycerides. Nature 2005; 433:523-7; PMID:15690042; http://dx.doi.org/10.1038/nature03253
16. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 2001; 410:1099-103; PMID:11323673; http://dx.doi.org/10.1038/35074106
17. Smith KD, Andersen-Nissen E, Hayashi F, Strobe K, Bergman MA, Barrett SL, et al. Toll-like receptor 5 recognizes a conserved site on flagellin required for protofilament formation and bacterial motility. Nat Immunol 2003; 4:1247-53; PMID:14625549; http://dx.doi.org/10.1038/ni1011
18. Lu J, Sun PD. The structure of the TLR5-flagellin complex: a new mode of pathogen detection, conserved receptor dimerization for signaling. Sci Signal 2012; 5:pe11; http://dx.doi.org/10.1126/scisignal.2002963
19. Yoon SI, Kurnasov O, Natarajan V, Hong M, Gudkov AV, Osterman AL, et al. Structural basis of TLR5-flagellin recognition and signaling. Science 2012; 335:859-64; PMID:22344444; http://dx.doi.org/10.1126/science.1215584
20. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, et al. A Toll-like receptor recognizes bacterial DNA. Nature 2000; 408:740-5; PMID:11130078; http://dx.doi.org/10.1038/35047123
21. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 2004; 303:1529-31; PMID:14976261; http://dx.doi.org/10.1126/science.1093616
22. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, et al. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 2004; 303:1526-9; PMID:14976262; http://dx.doi.org/10.1126/science.1093620
23. Hotz C, Bourquin C. Systemic cancer immunotherapy with Toll-like receptor 7 agonists: Timing is everything. Oncoimmunology 2012; 1:227-8; PMID:22720251; http://dx.doi.org/10.4161/onci.1.2.18169
24. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 2001; 413:732-8; PMID:11607032; http://dx.doi.org/10.1038/35099560
25. Choe J, Kelker MS, Wilson IA. Crystal structure of human toll-like receptor 3 (TLR3) ectodomain. Science 2005; 309:581-5; PMID:15961631; http://dx.doi.org/10.1126/science.1115253
26. Liu L, Botos I, Wang Y, Leonard JN, Shiloach J, Segal DM, et al. Structural basis of toll-like receptor 3 signaling with double-stranded RNA. Science 2008; 320:379-81; PMID:18420935; http://dx.doi.org/10.1126/science.1155406
27. Hennessy EJ, Parker AE, O'Neill LA. Targeting Toll-like receptors: emerging therapeutics? Nat Rev Drug Discov 2010; 9:293-307; PMID:20380038; http://dx.doi.org/10.1038/nrd3203
28. Jinushi M. The role of innate immune signals in antitumor immunity. Oncoimmunology 2012; 1:189-94; PMID:22720240; http://dx.doi.org/10.4161/onci.1.2.18495
29. Matzinger P. The danger model: a renewed sense of self. Science 2002; 296:301-5; PMID:11951032; http://dx.doi.org/10.1126/science.1071059
30. Chen GY, Nuñez G. Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol 2010; 10:826-37; PMID:21088683; http://dx.doi.org/10.1038/nri2873
31. Brenner C, Galluzzi L, Kepp O, Kroemer G. Decoding cell death signals in liver inflammation. J Hepatol 2013; In press; PMID:23567086; http://dx.doi.org/10.1016/j.jhep.2013.03.033
32. Galluzzi L, Kepp O, Kroemer G. Mitochondria: master regulators of danger signalling. Nat Rev Mol Cell Biol 2012; 13:780-8; PMID:23175281; http://dx.doi.org/10.1038/nrm3479
33. Asea A, Rehli M, Kabingu E, Boch JA, Bare O, Auron PE, et al. Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. J Biol Chem 2002; 277:15028-34; PMID:11836257; http://dx.doi.org/10.1074/jbc.M200497200
34. Kol A, Lichtman AH, Finberg RW, Libby P, Kurt-Jones EA. Cutting edge: heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. J Immunol 2000; 164:13-7; PMID:10604986
35. Liu-Bryan R, Pritzker K, Firestein GS, Terkeltaub R. TLR2 signaling in chondrocytes drives calcium pyrophosphate dihydrate and monosodium urate crystal-induced nitric oxide generation. J Immunol 2005; 174:5016-23; PMID:15814732
36. Guillot L, Balloy V, McCormack FX, Golenbock DT, Chignard M, Si-Tahar M. Cutting edge: the immunostimulatory activity of the lung surfactant protein-A involves Toll-like receptor 4. J Immunol 2002; 168:5989-92; PMID:12055204
37. Loser K, Vogl T, Voskort M, Lueken A, Kupas V, Nacken W, et al. The Toll-like receptor 4 ligands Mrp8 and Mrp14 are crucial in the development of autoreactive CD8+ T cells. Nat Med 2010; 16:713-7; PMID:20473308; http://dx.doi.org/10.1038/nm.2150
38. Vogl T, Tenbrock K, Ludwig S, Leukert N, Ehrhardt C, van Zoelen MA, et al. Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. Nat Med 2007; 13:1042-9; PMID:17767165; http://dx.doi.org/10.1038/nm1638
39. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol 2013; 31:51-72; PMID:23157435; http://dx.doi.org/10.1146/annurev-immunol-032712-100008
40. Kepp O, Galluzzi L, Martins I, Schlemmer F, Adjemian S, Michaud M, et al. Molecular determinants of immunogenic cell death elicited by anticancer chemotherapy. Cancer Metastasis Rev 2011; 30:61-9; PMID:21249425; http://dx.doi.org/10.1007/s10555-011-9273-4
41. Heijmans J, Büller NV, Muncan V, van den Brink GR. Rage mediated DAMP signaling in intestinal tumorigenesis. Oncoimmunology 2012; 1:1165-6; PMID:23170266; http://dx.doi.org/10.4161/onci.20929
42. Rakoff-Nahoum S, Medzhitov R. Toll-like receptors and cancer. Nat Rev Cancer 2009; 9:57-63; PMID:19052556; http://dx.doi.org/10.1038/nrc2541
43. Marques PE, Amaral SS, Pires DA, Nogueira LL, Soriani FM, Lima BH, et al. Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure. Hepatology 2012; 56:1971-82; PMID:22532075; http://dx.doi.org/10.1002/hep.25801
44. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature 2010; 464:104-7; PMID:20203610; http://dx.doi.org/10.1038/nature08780
45. Barton GM, Kagan JC, Medzhitov R. Intracellular localization of Toll-like receptor 9 prevents recognition of self DNA but facilitates access to viral DNA. Nat Immunol 2006; 7:49-56; PMID:16341217; http://dx.doi.org/10.1038/ni1280

46. Krieg AM. CpG motifs: the active ingredient in bacterial extracts? Nat Med 2003; 9:831-5; PMID:12835699; http://dx.doi.org/10.1038/nm0703-831
47. Lee CC, Avalos AM, Ploegh HL. Accessory molecules for Toll-like receptors and their function. Nat Rev Immunol 2012; 12:168-79; PMID:22301850
48. Barton GM, Kagan JC. A cell biological view of Toll-like receptor function: regulation through compartmentalization. Nat Rev Immunol 2009; 9:535-42; PMID:19556980; http://dx.doi.org/10.1038/nri2587
49. O'Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol 2007; 7:353-64; PMID:17457343; http://dx.doi.org/10.1038/nri2079
50. Galluzzi L, Vacchelli E, Eggermont A, Fridman WH, Galon J, Sautès-Fridman C, et al. Trial Watch: Experimental Toll-like receptor agonists for cancer therapy. Oncoimmunology 2012; 1:699-716; PMID:22934262; http://dx.doi.org/10.4161/onci.20696
51. Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny MV, et al. Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. Cell Death Differ 2012; 19:107-20; PMID:21760595; http://dx.doi.org/10.1038/cdd.2011.96
52. Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P. Immunogenic cell death and DAMPs in cancer therapy. Nat Rev Cancer 2012; 12:860-75; PMID:23151605; http://dx.doi.org/10.1038/nrc3380
53. Kono K, Mimura K. Immunogenic tumor cell death induced by chemoradiotherapy in a clinical setting. Oncoimmunology 2013; 2:e22197; PMID:23482346; http://dx.doi.org/10.4161/onci.22197
54. Apetoh L, Ghiringhelli F, Tesniere A, Criollo A, Ortiz C, Lidereau R, et al. The interaction between HMGB1 and TLR4 dictates the outcome of anticancer chemotherapy and radiotherapy. Immunol Rev 2007; 220:47-59; PMID:17979839; http://dx.doi.org/10.1111/j.1600-065X.2007.00573.x
55. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007; 13:1050-9; PMID:17704786; http://dx.doi.org/10.1038/nm1622
56. Chen T, Guo J, Han C, Yang M, Cao X. Heat shock protein 70, released from heat-stressed tumor cells, initiates antitumor immunity by inducing tumor cell chemokine production and activating dendritic cells via TLR4 pathway. J Immunol 2009; 182:1449-59; PMID:19155492
57. Hoffman ES, Smith RE, Renaud RC Jr. From the analyst's couch: TLR-targeted therapeutics. Nat Rev Drug Discov 2005; 4:879-80; PMID:16299917; http://dx.doi.org/10.1038/nrd1880
58. Standiford TJ, Keshamouni VG. Breaking the tolerance for tumor: Targeting negative regulators of TLR signaling. Oncoimmunology 2012; 1:340-5; PMID:22737610; http://dx.doi.org/10.4161/onci.18434
59. Tahara T, Arisawa T, Wang F, Shibata T, Nakamura M, Sakata M, et al. Toll-like receptor 2 -196 to 174del polymorphism influences the susceptibility of Japanese people to gastric cancer. Cancer Sci 2007; 98:1790-4; PMID:17711514; http://dx.doi.org/10.1111/j.1349-7006.2007.00590.x
60. Mollaki V, Georgiadis T, Tassidou A, Ioannou M, Daniil Z, Koutsokera A, et al. Polymorphisms and haplotypes in TLR9 and MYD88 are associated with the development of Hodgkin's lymphoma: a candidate-gene association study. J Hum Genet 2009; 54:655-9; PMID:19745833; http://dx.doi.org/10.1038/jhg.2009.90
61. Gast A, Bermejo JL, Claus R, Brandt A, Weires M, Weber A, et al. Association of inherited variation in Toll-like receptor genes with malignant melanoma susceptibility and survival. PLoS One 2011; 6:e24370; PMID:21931695; http://dx.doi.org/10.1371/journal.pone.0024370
62. Guirado M, Gil H, Saenz-Lopez P, Reinboth J, Garrido F, Cozar JM, et al. Association between C13ORF31, NOD2, RIPK2 and TLR10 polymorphisms and urothelial bladder cancer. Hum Immunol 2012; 73:668-72; PMID:22504414; http://dx.doi.org/10.1016/j.humimm.2012.03.006
63. Junjie X, Songyao J, Minmin S, Yanyan S, Baiyong S, Xiaxing D, et al. The association between Toll-like receptor 2 single-nucleotide polymorphisms and hepatocellular carcinoma susceptibility. BMC Cancer 2012; 12:57; PMID:22309608; http://dx.doi.org/10.1186/1471-2407-12-57
64. Carvalho A, Cunha C, Almeida AJ, Osório NS, Saraiva M, Teixeira-Coelho M, et al. The rs5743836 polymorphism in TLR9 confers a population-based increased risk of non-Hodgkin lymphoma. Genes Immun 2012; 13:197-201; PMID:21866115; http://dx.doi.org/10.1038/gene.2011.59
65. Kim MK, Park SW, Kim SK, Park HJ, Eun YG, Kwon KH, et al. Association of Toll-like receptor 2 polymorphisms with papillary thyroid cancer and clinicopathologic features in a Korean population. J Korean Med Sci 2012; 27:1333-8; PMID:23166414; http://dx.doi.org/10.3346/jkms.2012.27.11.1333
66. Yang ZH, Dai Q, Gu YJ, Guo QX, Gong L. Cytokine and chemokine modification by Toll-like receptor polymorphisms is associated with nasopharyngeal carcinoma. Cancer Sci 2012; 103:653-8; PMID:22320262; http://dx.doi.org/10.1111/j.1349-7006.2012.02210.x
67. Resler AJ, Malone KE, Johnson LG, Malkki M, Petersdorf EW, McKnight B, et al. Genetic variation in TLR or NFkappaB pathways and the risk of breast cancer: a case-control study. BMC Cancer 2013; 13:219; PMID:23634849; http://dx.doi.org/10.1186/1471-2407-13-219
68. Roszak A, Lianeri M, Sowińska A, Jagodziński PP. Involvement of Toll-like Receptor 9 polymorphism in cervical cancer development. Mol Biol Rep 2012; 39:8425-30; PMID:22714906; http://dx.doi.org/10.1007/s11033-012-1695-8
69. Agúndez JA, García-Martín E, Devesa MJ, Carballo M, Martínez C, Lee-Brunner A, et al. Polymorphism of the TLR4 gene reduces the risk of hepatitis C virus-induced hepatocellular carcinoma. Oncology 2012; 82:35-40; PMID:22286521; http://dx.doi.org/10.1159/000335606
70. Castaño-Rodríguez N, Kaakoush NO, Goh KL, Fock KM, Mitchell HM. The role of TLR2, TLR4 and CD14 genetic polymorphisms in gastric carcinogenesis: a case-control study and meta-analysis. PLoS One 2013; 8:e60327; PMID:23565226; http://dx.doi.org/10.1371/journal.pone.0060327
71. Kim HJ, Bae JS, Chang IH, Kim KD, Lee J, Shin HD, et al. Sequence variants of Toll-like receptor 4 (TLR4) and the risk of prostate cancer in Korean men. World J Urol 2012; 30:225-32; PMID:21553150; http://dx.doi.org/10.1007/s00345-011-0690-3
72. Zhang K, Zhou B, Wang Y, Rao L, Zhang L. The TLR4 gene polymorphisms and susceptibility to cancer: a systematic review and meta-analysis. Eur J Cancer 2013; 49:946-54; PMID:23084080; http://dx.doi.org/10.1016/j.ejca.2012.09.022
73. Nischalke HD, Coenen M, Berger C, Aldenhoff K, Müller T, Berg T, et al. The toll-like receptor 2 (TLR2) -196 to -174 del/ins polymorphism affects viral loads and susceptibility to hepatocellular carcinoma in chronic hepatitis C. Int J Cancer 2012; 130:1470-5; PMID:21500195; http://dx.doi.org/10.1002/ijc.26143
74. Wang X, Li J, Xie W, Zhang W, Chang Y. Toll-like receptor 2 gene polymorphisms and cancer susceptibility: A meta-analysis. Neoplasma 2013; 60:459-67; PMID:23581420; http://dx.doi.org/10.4149/neo_2013_060
75. Theodoropoulos GE, Saridakis V, Karantanos T, Michalopoulos NV, Zagouri F, Kontogianni P, et al. Toll-like receptors gene polymorphisms may confer increased susceptibility to breast cancer development. Breast 2012; 21:534-8; PMID:22560646; http://dx.doi.org/10.1016/j.breast.2012.04.001
76. Zou TH, Wang ZH, Fang JY. Positive association between Toll-like receptor 4 gene +896A/G polymorphism and susceptibility to gastric carcinogenesis: a meta-analysis. Tumour Biol 2013; In press; PMID:23592020; http://dx.doi.org/10.1007/s13277-013-0795-y
77. Chew V, Tow C, Huang C, Bard-Chapeau E, Copeland NG, Jenkins NA, et al. Toll-like receptor 3 expressing tumor parenchyma and infiltrating natural killer cells in hepatocellular carcinoma patients. J Natl Cancer Inst 2012; 104:1796-807; PMID:23197495; http://dx.doi.org/10.1093/jnci/djs436
78. Galluzzi L, Senovilla L, Zitvogel L, Kroemer G. The secret ally: immunostimulation by anticancer drugs. Nat Rev Drug Discov 2012; 11:215-33; PMID:22301798; http://dx.doi.org/10.1038/nrd3626
79. Ahmed A, Redmond HP, Wang JH. Links between Toll-like receptor 4 and breast cancer. Oncoimmunology 2013; 2:e22945; PMID:23526132; http://dx.doi.org/10.4161/onci.22945
80. Tittarelli A, González FE, Pereda C, Mora G, Muñoz L, Saffie C, et al. Toll-like receptor 4 gene polymorphism influences dendritic cell in vitro function and clinical outcomes in vaccinated melanoma patients. Cancer Immunol Immunother 2012; 61:2067-77; PMID:22552381; http://dx.doi.org/10.1007/s00262-012-1268-7
81. Eiró N, González L, González LO, Andicoechea A, Fernández-Díaz M, Altadill A, et al. Study of the expression of toll-like receptors in different histological types of colorectal polyps and their relationship with colorectal cancer. J Clin Immunol 2012; 32:848-54; PMID:22371291; http://dx.doi.org/10.1007/s10875-012-9666-3
82. Eiró N, Ovies C, Fernandez-Garcia B, Álvarez-Cuesta CC, González L, González LO, et al. Expression of TLR3, 4, 7 and 9 in cutaneous malignant melanoma: relationship with clinicopathological characteristics and prognosis. Arch Dermatol Res 2013; 305:59-67; PMID:23179584; http://dx.doi.org/10.1007/s00403-012-1300-y
83. Femia AP, Swidsinski A, Dolara P, Salvadori M, Amedei A, Caderni G. Mucin depleted foci, colonic preneoplastic lesions lacking Muc2, show up-regulation of Tlr2 but not bacterial infiltration. PLoS One 2012; 7:e29918; PMID:22242189; http://dx.doi.org/10.1371/journal.pone.0029918
84. Hagström J, Heikkilä A, Siironen P, Louhimo J, Heiskanen I, Mäenpää H, et al. TLR-4 expression and decrease in chronic inflammation: indicators of aggressive follicular thyroid carcinoma. J Clin Pathol 2012; 65:333-8; PMID:22267982; http://dx.doi.org/10.1136/jclinpath-2011-200402
85. Huan P, Maosheng T, Zhiqian H, Long C, Xiaojun Y. TLR4 expression in normal gallbladder, chronic cholecystitis and gallbladder carcinoma. Hepatogastroenterology 2012; 59:42-6; PMID:22251522
86. Leng L, Jiang T, Zhang Y. TLR9 expression is associated with prognosis in patients with glioblastoma multiforme. J Clin Neurosci 2012; 19:75-80; PMID:22169598; http://dx.doi.org/10.1016/j.jocn.2011.03.037

87. Tuomela J, Sandholm J, Karihtala P, Ilvesaro J, Vuopala KS, Kauppila JH, et al. Low TLR9 expression defines an aggressive subtype of triple-negative breast cancer. Breast Cancer Res Treat 2012; 135:481-93; PMID:22847512; http://dx.doi.org/10.1007/s10549-012-2181-7

88. Kauppila JH, Mattila AE, Karttunen TJ, Salo T. Toll-like receptor 5 (TLR5) expression is a novel predictive marker for recurrence and survival in squamous cell carcinoma of the tongue. Br J Cancer 2013; 108:638-43; PMID:23287987; http://dx.doi.org/10.1038/bjc.2012.589

89. Ehsan N, Murad S, Ashiq T, Mansoor MU, Gul S, Khalid S, et al. Significant correlation of TLR4 expression with the clinicopathological features of invasive ductal carcinoma of the breast. Tumour Biol 2013; 34:1053-9; PMID:23338716; http://dx.doi.org/10.1007/s13277-013-0645-y

90. Chuang HC, Huang CC, Chien CY, Chuang JH. Toll-like receptor 3-mediated tumor invasion in head and neck cancer. Oral Oncol 2012; 48:226-32; PMID:22070917; http://dx.doi.org/10.1016/j.oraloncology.2011.10.008

91. Jinesh G G, Kamat AM. Redirecting neutrophils against bladder cancer cells by BCG and Smac mimetic combination. Oncoimmunology 2012; 1:1161-2; PMID:23170264; http://dx.doi.org/10.4161/onci.20928

92. Paavonen J, Naud P, Salmerón J, Wheeler CM, Chow SN, Apter D, et al.; HPV PATRICIA Study Group. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 2009; 374:301-14; PMID:19586656; http://dx.doi.org/10.1016/S0140-6736(09)61248-4

93. Lehtinen M, Paavonen J. Sound efficacy of prophylactic HPV vaccination: Basics and implications. Oncoimmunology 2012; 1:995-6; PMID:23162784; http://dx.doi.org/10.4161/onci.20011

94. Heldwein KA, Liang MD, Andresen TK, Thomas KE, Marty AM, Cuesta N, et al. TLR2 and TLR4 serve distinct roles in the host immune response against Mycobacterium bovis BCG. J Leukoc Biol 2003; 74:277-86; PMID:12885945; http://dx.doi.org/10.1189/jlb.0103026

95. Uehori J, Matsumoto M, Tsuji S, Akazawa T, Takeuchi O, Akira S, et al. Simultaneous blocking of human Toll-like receptors 2 and 4 suppresses myeloid dendritic cell activation induced by Mycobacterium bovis bacillus Calmette-Guérin peptidoglycan. Infect Immun 2003; 71:4238-49; PMID:12874299; http://dx.doi.org/10.1128/IAI.71.8.4238-4249.2003

96. Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol 2002; 3:196-200; PMID:11812998; http://dx.doi.org/10.1038/ni758

97. Holcmann M, Drobits B, Sibilia M. How imiquimod licenses plasmacytoid dendritic cells to kill tumors. Oncoimmunology 2012; 1:1661-3; PMID:23264929; http://dx.doi.org/10.4161/onci.22033

98. Nauts HC, Swift WE, Coley BL. The treatment of malignant tumors by bacterial toxins as developed by the late William B. Coley, M.D., reviewed in the light of modern research. Cancer Res 1946; 6:205-16; PMID:21018724

99. Maletzki C, Klier U, Obst W, Kreikemeyer B, Linnebacher M. Reevaluating the concept of treating experimental tumors with a mixed bacterial vaccine: Coley's Toxin. Clin Dev Immunol 2012; 2012:230625; PMID:23193416; http://dx.doi.org/10.1155/2012/230625

100. Thotathil Z, Jameson MB. Early experience with novel immunomodulators for cancer treatment. Expert Opin Investig Drugs 2007; 16:1391-403; PMID:17714025; http://dx.doi.org/10.1517/13543784.16.9.1391

101. Oblak A, Jerala R. Toll-like receptor 4 activation in cancer progression and therapy. Clin Dev Immunol 2011; 2011:609579; PMID:22110526; http://dx.doi.org/10.1155/2011/609579

102. Ito T, Ando H, Suzuki T, Ogura T, Hotta K, Imamura Y, et al. Identification of a primary target of thalidomide teratogenicity. Science 2010; 327:1345-50; PMID:20223979; http://dx.doi.org/10.1126/science.1177319

103. Vacchelli E, Galluzzi L, Eggermont A, Fridman WH, Galon J, Sautès-Fridman C, et al. Trial watch: FDA-approved Toll-like receptor agonists for cancer therapy. Oncoimmunology 2012; 1:894-907; PMID:23162757; http://dx.doi.org/10.4161/onci.20931

104. Vacchelli E, Eggermont A, Fridman WH, Galon J, Tartour E, Zitvogel L, et al. Trial Watch: Adoptive cell transfer for anticancer immunotherapy. OncoImmunology 2013; 2:e24238; http://dx.doi.org/10.4161/onci.24238

105. Galluzzi L, Vacchelli E, Eggermont A, Fridman WH, Galon J, Sautès-Fridman C, et al. Trial Watch: Adoptive cell transfer immunotherapy. Oncoimmunology 2012; 1:306-15; PMID:22737606; http://dx.doi.org/10.4161/onci.19549

106. Menger L, Vacchelli E, Kepp O, Eggermont A, Tartour E, Zitvogel L, et al. Trial watch: Cardiac glycosides and cancer therapy. Oncoimmunology 2013; 2:e23082; PMID:23525565; http://dx.doi.org/10.4161/onci.23082

107. Vacchelli E, Galluzzi L, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, et al. Trial watch: Chemotherapy with immunogenic cell death inducers. Oncoimmunology 2012; 1:179-88; PMID:22720239; http://dx.doi.org/10.4161/onci.1.2.19026

108. Vacchelli E, Senovilla L, Eggermont A, Fridman WH, Galon J, Zitvogel L, et al. Trial watch: Chemotherapy with immunogenic cell death inducers. Oncoimmunology 2013; 2:e23510; PMID:23687621; http://dx.doi.org/10.4161/onci.23510

109. Galluzzi L, Senovilla L, Vacchelli E, Eggermont A, Fridman WH, Galon J, et al. Trial watch: Dendritic cell-based interventions for cancer therapy. Oncoimmunology 2012; 1:1111-34; PMID:23170259; http://dx.doi.org/10.4161/onci.21494

110. Senovilla L, Vacchelli E, Garcia P, Eggermont A, Fridman WH, Galon J, et al. Trial watch: DNA vaccines for cancer therapy. Oncoimmunology 2013; 2:e23803; PMID:23734328; http://dx.doi.org/10.4161/onci.23803

111. Vacchelli E, Eggermont A, Fridman WH, Galon J, Zitvogel L, Kroemer G, et al. Trial Watch: Immunostimulatory cytokines. Oncoimmunology 2013; 2: In press; http://dx.doi.org/10.146.onci.24850

112. Vacchelli E, Galluzzi L, Eggermont A, Galon J, Tartour E, Zitvogel L, et al. Trial Watch: Immunostimulatory cytokines. Oncoimmunology 2012; 1:493-506; PMID:22754768; http://dx.doi.org/10.4161/onci.20459

113. Galluzzi L, Vacchelli E, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, et al. Trial Watch: Monoclonal antibodies in cancer therapy. Oncoimmunology 2012; 1:28-37; PMID:22720209; http://dx.doi.org/10.4161/onci.1.1.17938

114. Vacchelli E, Eggermont A, Galon J, Sautès-Fridman C, Zitvogel L, Kroemer G, et al. Trial watch: Monoclonal antibodies in cancer therapy. Oncoimmunology 2013; 2:e22789; PMID:23482847; http://dx.doi.org/10.4161/onci.22789

115. Vacchelli E, Eggermont A, Sautès-Fridman C, Galon J, Zitvogel L, Kroemer G, et al. Trial Watch: Oncolytic viruses for cancer therapy. OncoImmunology 2013; 2:e24612; http://dx.doi.org/10.4161/onci.24612

116. Vacchelli E, Martins I, Eggermont A, Fridman WH, Galon J, Sautès-Fridman C, et al. Trial watch: Peptide vaccines in cancer therapy. Oncoimmunology 2012; 1:1557-76; PMID:23264902; http://dx.doi.org/10.4161/onci.22428

117. Senovilla L, Vacchelli E, Galon J, Adjemian S, Eggermont A, Fridman WH, et al. Trial watch: Prognostic and predictive value of the immune infiltrate in cancer. Oncoimmunology 2012; 1:1323-43; PMID:23243596; http://dx.doi.org/10.4161/onci.22009

118. Gontero P, Oderda M, Mehnert A, Gurioli A, Marson F, Lucca I, et al. The impact of intravesical gemcitabine and 1/3 dose Bacillus Calmette-Guerin instillation therapy on the quality of life in patients with non-muscle invasive bladder cancer: results of a prospective, randomized, phase II trial. J Urol 2013; In press; PMID:23545101; http://dx.doi.org/10.1016/j.juro.2013.03.097

119. Gülpinar O, Halilioglu AH, Gökçe MI, Görür C, Baltaci S. The value of perioperative mitomycin C instillation in improving subsequent bacillus calmette-guerin instillation efficacy in intermediate and high-risk patients with non-muscle invasive bladder cancer: a prospective randomized study. Int Braz J Urol 2012; 38:474-9; PMID:22951160

120. Kanagawa Urological Research Group (KURG). A 2-week maintenance regimen of intravesical instillation of bacillus Calmette-Guerin is safe, adherent and effective in patients with non-muscle-invasive bladder cancer: a prospective, multicenter phase II clinical trial. Jpn J Clin Oncol 2012; 42:813-9; PMID:22761255; http://dx.doi.org/10.1093/jjco/hys097

121. Oddens J, Brausi M, Sylvester R, Bono A, van de Beek C, van Andel G, et al. Final results of an EORTC-GU cancers group randomized study of maintenance bacillus Calmette-Guérin in intermediate- and high-risk Ta, T1 papillary carcinoma of the urinary bladder: one-third dose versus full dose and 1 year versus 3 years of maintenance. Eur Urol 2013; 63:462-72; PMID:23141049; http://dx.doi.org/10.1016/j.eururo.2012.10.039

122. Kamat AM, Dickstein RJ, Messetti F, Anderson R, Pretzsch SM, Gonzalez GN, et al. Use of fluorescence in situ hybridization to predict response to bacillus Calmette-Guérin therapy for bladder cancer: results of a prospective trial. J Urol 2012; 187:862-7; PMID:22245325; http://dx.doi.org/10.1016/j.juro.2011.10.144

123. Pedersen C, Breindahl M, Aggarwal N, Berglund J, Oroszlán G, Silfverdal SA, et al. Randomized trial: immunogenicity and safety of coadministered human papillomavirus-16/18 AS04-adjuvanted vaccine and combined hepatitis A and B vaccine in girls. J Adolesc Health 2012; 50:38-46; PMID:22188832; http://dx.doi.org/10.1016/j.jadohealth.2011.10.009

124. Kensil CR, Kammer R. QS-21: a water-soluble triterpene glycoside adjuvant. Expert Opin Investig Drugs 1998; 7:1475-82; PMID:15992044; http://dx.doi.org/10.1517/13543784.7.9.1475

125. Hamilton E, Blackwell K, Hobeika AC, Clay TM, Broadwater G, Ren XR, et al. Phase 1 clinical trial of HER2-specific immunotherapy with concomitant HER2 kinase inhibition [corrected]. J Transl Med 2012; 10:28; PMID:22325452; http://dx.doi.org/10.1186/1479-5876-10-28

126. Moy B, Kirkpatrick P, Kar S, Goss P. Lapatinib. Nat Rev Drug Discov 2007; 6:431-2; PMID:17633789; http://dx.doi.org/10.1038/nrd2332

127. Osiecka B, Jurczyszyn K, Ziółkowski P. The application of Levulan-based photodynamic therapy with imiquimod in the treatment of recurrent basal cell carcinoma. Med Sci Monit 2012; 18:PI5-9; PMID:22293891; http://dx.doi.org/10.12659/MSM.882449

128. Serra-Guillén C, Nagore E, Hueso L, Traves V, Messeguer F, Sanmartín O, et al. A randomized pilot comparative study of topical methyl aminolevulinate photodynamic therapy versus imiquimod 5% versus sequential application of both therapies in immunocompetent patients with actinic keratosis: clinical and histologic outcomes. J Am Acad Dermatol 2012; 66:e131-7; PMID:22226430; http://dx.doi.org/10.1016/j.jaad.2011.11.933

129. van der Geer S, Martens J, van Roij J, Brand E, Ostertag JU, Verhaegh ME, et al. Imiquimod 5% cream as pretreatment of Mohs micrographic surgery for nodular basal cell carcinoma in the face: a prospective randomized controlled study. Br J Dermatol 2012; 167:110-5; PMID:22385074; http://dx.doi.org/10.1111/j.1365-2133.2012.10924.x
130. Narayan R, Nguyen H, Bentow JJ, Moy L, Lee DK, Greger S, et al. Immunomodulation by imiquimod in patients with high-risk primary melanoma. J Invest Dermatol 2012; 132:163-9; PMID:21850019; http://dx.doi.org/10.1038/jid.2011.247
131. Goldinger SM, Dummer R, Baumgaertner P, Mihic-Probst D, Schwarz K, Hammann-Haenni A, et al. Nano-particle vaccination combined with TLR-7 and -9 ligands triggers memory and effector $CD8^+$ T-cell responses in melanoma patients. Eur J Immunol 2012; 42:3049-61; PMID:22806397; http://dx.doi.org/10.1002/eji.201142361
132. Adams S, Kozhaya L, Martiniuk F, Meng TC, Chiriboga L, Liebes L, et al. Topical TLR7 agonist imiquimod can induce immune-mediated rejection of skin metastases in patients with breast cancer. Clin Cancer Res 2012; 18:6748-57; PMID:22767669; http://dx.doi.org/10.1158/1078-0432.CCR-12-1149
133. Florin V, Desmedt E, Vercambre-Darras S, Mortier L. Topical treatment of cutaneous metastases of malignant melanoma using combined imiquimod and 5-fluorouracil. Invest New Drugs 2012; 30:1641-5; PMID:21748297; http://dx.doi.org/10.1007/s10637-011-9717-2
134. Fenoglio D, Traverso P, Parodi A, Tomasello L, Negrini S, Kalli F, et al. A multi-peptide, dual-adjuvant telomerase vaccine (GX301) is highly immunogenic in patients with prostate and renal cancer. Cancer Immunol Immunother 2013; 62:1041-52; PMID:23591981; http://dx.doi.org/10.1007/s00262-013-1415-9
135. Grimm C, Polterauer S, Natter C, Rahhal J, Hefler L, Tempfer CB, et al. Treatment of cervical intraepithelial neoplasia with topical imiquimod: a randomized controlled trial. Obstet Gynecol 2012; 120:152-9; PMID:22914404; http://dx.doi.org/10.1097/AOG.0b013e31825bc6e8
136. Richel O, de Vries HJ, van Noesel CJ, Dijkgraaf MG, Prins JM. Comparison of imiquimod, topical fluorouracil, and electrocautery for the treatment of anal intraepithelial neoplasia in HIV-positive men who have sex with men: an open-label, randomised controlled trial. Lancet Oncol 2013; 14:346-53; PMID:23499546; http://dx.doi.org/10.1016/S1470-2045(13)70067-6
137. Falke J, Lammers RJ, Arentsen HC, Ravic M, Pozzi R, Cornel EB, et al. Results of a Phase 1 Dose Escalation Study of Intravesical TMX-101 in Patients with Nonmuscle Invasive Bladder Cancer. J Urol 2013; 189:2077-82; PMID:23206424; http://dx.doi.org/10.1016/j.juro.2012.11.150
138. Okamoto H, Shoin S, Koshimura S, Shimizu R. Studies on the anticancer and streptolysin S-forming abilities of hemolytic streptococci. Jpn J Microbiol 1967; 11:323-6; PMID:4875331
139. Endo H, Saito T, Kenjo A, Hoshino M, Terashima M, Sato T, et al. Phase I trial of preoperative intratumoral injection of immature dendritic cells and OK-432 for resectable pancreatic cancer patients. J Hepatobiliary Pancreat Sci 2012; 19:465-75; PMID:21983893; http://dx.doi.org/10.1007/s00534-011-0457-7
140. Galluzzi L, Senovilla L, Vitale I, Michels J, Martins I, Kepp O, et al. Molecular mechanisms of cisplatin resistance. Oncogene 2012; 31:1869-83; PMID:21892204; http://dx.doi.org/10.1038/onc.2011.384
141. Chen WJ, Yuan SF, Yan QY, Xiong JP, Wang SM, Zheng WE, et al. Intrapleural chemo- and hyperthermotherapies for malignant pleural effusion: a randomized prospective study. Cancer Invest 2012; 30:126-30; PMID:22148972; http://dx.doi.org/10.3109/07357907.2011.633292
142. Kakimi K, Isobe M, Uenaka A, Wada H, Sato E, Doki Y, et al. A phase I study of vaccination with NY-ESO-1f peptide mixed with Picibanil OK-432 and Montanide ISA-51 in patients with cancers expressing the NY-ESO-1 antigen. Int J Cancer 2011; 129:2836-46; PMID:21448901; http://dx.doi.org/10.1002/ijc.25955
143. Eikawa S, Kakimi K, Isobe M, Kuzushima K, Luescher I, Ohue Y, et al. Induction of CD8 T-cell responses restricted to multiple HLA class I alleles in a cancer patient by immunization with a 20-mer NY-ESO-1f (NY-ESO-1 91-110) peptide. Int J Cancer 2013; 132:345-54; PMID:22729530; http://dx.doi.org/10.1002/ijc.27682
144. Brandenburg K, Lindner B, Schromm A, Koch MH, Bauer J, Merkli A, et al. Physicochemical characteristics of triacyl lipid A partial structure OM-174 in relation to biological activity. Eur J Biochem 2000; 267:3370-7; PMID:10824125; http://dx.doi.org/10.1046/j.1432-1327.2000.01370.x
145. Isambert N, Fumoleau P, Paul C, Ferrand C, Zanetta S, Bauer J, et al. Phase I study of OM-174, a lipid A analogue, with assessment of immunological response, in patients with refractory solid tumors. BMC Cancer 2013; 13:172; PMID:23547558; http://dx.doi.org/10.1186/1471-2407-13-172
146. Yuksel ZS, Buber E, Kocagoz T, Alp A, Saribas Z, Acan NL. Mycobacterial strains that stimulate the immune system most efficiently as candidates for the treatment of bladder cancer. J Mol Microbiol Biotechnol 2011; 20:24-8; PMID:21335976; http://dx.doi.org/10.1159/000324331
147. Stebbing J, Dalgleish A, Gifford-Moore A, Martin A, Gleeson C, Wilson G, et al. An intra-patient placebo-controlled phase I trial to evaluate the safety and tolerability of intradermal IMM-101 in melanoma. Ann Oncol 2012; 23:1314-9; PMID:21930686; http://dx.doi.org/10.1093/annonc/mdr363
148. Levy HB, Baer G, Baron S, Buckler CE, Gibbs CJ, Iadarola MJ, et al. A modified polyriboinosinic-polyribocytidylic acid complex that induces interferon in primates. J Infect Dis 1975; 132:434-9; PMID:810520; http://dx.doi.org/10.1093/infdis/132.4.434
149. Sabbatini P, Tsuji T, Ferran L, Ritter E, Sedrak C, Tuballes K, et al. Phase I trial of overlapping long peptides from a tumor self-antigen and poly-ICLC shows rapid induction of integrated immune response in ovarian cancer patients. Clin Cancer Res 2012; 18:6497-508; PMID:23032745; http://dx.doi.org/10.1158/1078-0432.CCR-12-2189
150. Weigel BJ, Cooley S, DeFor T, Weisdorf DJ, Panoskaltsis-Mortari A, Chen W, et al. Prolonged subcutaneous administration of 852A, a novel systemic toll-like receptor 7 agonist, to activate innate immune responses in patients with advanced hematologic malignancies. Am J Hematol 2012; 87:953-6; PMID:22718533; http://dx.doi.org/10.1002/ajh.23280
151. Agrawal S, Kandimalla ER. Synthetic agonists of Toll-like receptors 7, 8 and 9. Biochem Soc Trans 2007; 35:1461-7; PMID:18031246; http://dx.doi.org/10.1042/BST0351461
152. Kandimalla ER, Bhagat L, Li Y, Yu D, Wang D, Cong YP, et al. Immunomodulatory oligonucleotides containing a cytosine-phosphate-2'-deoxy-7-deazaguanosine motif as potent toll-like receptor 9 agonists. Proc Natl Acad Sci U S A 2005; 102:6925-30; PMID:15860583; http://dx.doi.org/10.1073/pnas.0501729102
153. Graham J, Muhsin M, Kirkpatrick P. Cetuximab. Nat Rev Drug Discov 2004; 3:549-50; PMID:15272498; http://dx.doi.org/10.1038/nrd1445
154. Machiels JP, Kaminsky MC, Keller U, Brümmendorf TH, Goddemeier T, Forssmann U, et al. Phase Ib trial of the Toll-like receptor 9 agonist IMO-2055 in combination with 5-fluorouracil, cisplatin, and cetuximab as first-line palliative treatment in patients with recurrent/metastatic squamous cell carcinoma of the head and neck. Invest New Drugs 2013; PMID:23397499; http://dx.doi.org/10.1007/s10637-013-9933-z
155. Murad YM, Clay TM, Lyerly HK, Morse MA. CPG-7909 (PF-3512676, ProMune): toll-like receptor-9 agonist in cancer therapy. Expert Opin Biol Ther 2007; 7:1257-66; PMID:17696823; http://dx.doi.org/10.1517/14712598.7.8.1257
156. Ribas A. Clinical development of the anti-CTLA-4 antibody tremelimumab. Semin Oncol 2010; 37:450-4; PMID:21074059; http://dx.doi.org/10.1053/j.seminoncol.2010.09.010
157. Waitz R, Fassò M, Allison JP. CTLA-4 blockade synergizes with cryoablation to mediate tumor rejection. Oncoimmunology 2012; 1:544-6; PMID:22754781; http://dx.doi.org/10.4161/onci.19442
158. Millward M, Underhill C, Lobb S, McBurnie J, Meech SJ, Gomez-Navarro J, et al. Phase I study of tremelimumab (CP-675 206) plus PF-3512676 (CPG 7909) in patients with melanoma or advanced solid tumours. Br J Cancer 2013; 108:1998-2004; PMID:23652314; http://dx.doi.org/10.1038/bjc.2013.227
159. Kim YH, Gratzinger D, Harrison C, Brody JD, Czerwinski DK, Ai WZ, et al. In situ vaccination against mycosis fungoides by intratumoral injection of a TLR9 agonist combined with radiation: a phase 1/2 study. Blood 2012; 119:355-63; PMID:22045986; http://dx.doi.org/10.1182/blood-2011-05-355222
160. Dovedi SJ, Melis MH, Wilkinson RW, Adlard AL, Stratford IJ, Honeychurch J, et al. Systemic delivery of a TLR7 agonist in combination with radiation primes durable antitumor immune responses in mouse models of lymphoma. Blood 2013; 121:251-9; PMID:23086756; http://dx.doi.org/10.1182/blood-2012-05-432393
161. Betting DJ, Hurvitz SA, Steward KK, Yamada RE, Kafi K, van Rooijen N, et al. Combination of cyclophosphamide, rituximab, and intratumoral CpG oligodeoxynucleotide successfully eradicates established B cell lymphoma. J Immunother 2012; 35:534-43; PMID:22892450; http://dx.doi.org/10.1097/CJI.0b013e318261e679
162. Ding X, Bian G, Leigh ND, Qiu J, McCarthy PL, Liu H, et al. A TLR5 agonist enhances CD8(+) T cell-mediated graft-versus-tumor effect without exacerbating graft-versus-host disease. J Immunol 2012; 189:4719-27; PMID:23045613; http://dx.doi.org/10.4049/jimmunol.1201206
163. Li X, Liu D, Liu X, Jiang W, Zhou W, Yan W, et al. CpG ODN107 potentiates radiosensitivity of human glioma cells via TLR9-mediated NF-κB activation and NO production. Tumour Biol 2012; 33:1607-18; PMID:22739939; http://dx.doi.org/10.1007/s13277-012-0416-1
164. Dewan MZ, Vanpouille-Box C, Kawashima N, DiNapoli S, Babb JS, Formenti SC, et al. Synergy of topical toll-like receptor 7 agonist with radiation and low-dose cyclophosphamide in a mouse model of cutaneous breast cancer. Clin Cancer Res 2012; 18:6668-78; PMID:23048078; http://dx.doi.org/10.1158/1078-0432.CCR-12-0984
165. Naseemuddin M, Iqbal A, Nasti TH, Ghandhi JL, Kapadia AD, Yusuf N. Cell mediated immune responses through TLR4 prevents DMBA-induced mammary carcinogenesis in mice. Int J Cancer 2012; 130:765-74; PMID:21455984; http://dx.doi.org/10.1002/ijc.26100
166. Garaude J, Kent A, van Rooijen N, Blander JM. Simultaneous targeting of toll- and nod-like receptors induces effective tumor-specific immune responses. Sci Transl Med 2012; 4:20ra16; PMID:22323829; http://dx.doi.org/10.1126/scitranslmed.3002868
167. Peng J, Tsang JY, Li D, Niu N, Ho DH, Lau KF, et al. Inhibition of TGF-β signaling in combination with TLR7 ligation re-programs a tumoricidal phenotype in tumor-associated macrophages. Cancer Lett 2013; 331:239-49; PMID:23318200; http://dx.doi.org/10.1016/j.canlet.2013.01.001

168. Lu H, Dietsch GN, Matthews MA, Yang Y, Ghanekar S, Inokuma M, et al. VTX-2337 is a novel TLR8 agonist that activates NK cells and augments ADCC. Clin Cancer Res 2012; 18:499-509; PMID:22128302; http://dx.doi.org/10.1158/1078-0432.CCR-11-1625
169. Stephenson RM, Lim CM, Matthews M, Dietsch G, Hershberg R, Ferris RL. TLR8 stimulation enhances cetuximab-mediated natural killer cell lysis of head and neck cancer cells and dendritic cell cross-priming of EGFR-specific CD8(+) T cells. Cancer Immunol Immunother 2013; In press; PMID:23685782; http://dx.doi.org/10.1007/s00262-013-1437-3
170. Núñez NG, Andreani V, Crespo MI, Nocera DA, Breser ML, Morón G, et al. IFNβ produced by TLR4-activated tumor cells is involved in improving the antitumoral immune response. Cancer Res 2012; 72:592-603; PMID:22139376; http://dx.doi.org/10.1158/0008-5472.CAN-11-0534
171. Van De Voort TJ, Felder MA, Yang RK, Sondel PM, Rakhmilevich AL. Intratumoral delivery of low doses of anti-CD40 mAb combined with monophosphoryl lipid a induces local and systemic antitumor effects in immunocompetent and T cell-deficient mice. J Immunother 2013; 36:29-40; PMID:23211623; http://dx.doi.org/10.1097/CJI.0b013e3182780f61
172. Xiao H, Peng Y, Hong Y, Huang L, Guo ZS, Bartlett DL, et al. Local Administration of TLR Ligands Rescues the Function of Tumor-Infiltrating CD8 T Cells and Enhances the Antitumor Effect of Lentivector Immunization. J Immunol 2013; 190:5866-73; PMID:23610140; http://dx.doi.org/10.4049/jimmunol.1203470
173. Roy A, Singh MS, Upadhyay P, Bhaskar S. Nanoparticle mediated co-delivery of paclitaxel and a TLR-4 agonist results in tumor regression and enhanced immune response in the tumor microenvironment of a mouse model. Int J Pharm 2013; 445:171-80; PMID:23376226; http://dx.doi.org/10.1016/j.ijpharm.2013.01.045
174. Marshall NA, Galvin KC, Corcoran AM, Boon L, Higgs R, Mills KH. Immunotherapy with PI3K inhibitor and Toll-like receptor agonist induces IFN-γ+IL-17+ polyfunctional T cells that mediate rejection of murine tumors. Cancer Res 2012; 72:581-91; PMID:22158905; http://dx.doi.org/10.1158/0008-5472.CAN-11-0307
175. Cerullo V, Diaconu I, Romano V, Hirvinen M, Ugolini M, Escutenaire S, et al. An oncolytic adenovirus enhanced for toll-like receptor 9 stimulation increases antitumor immune responses and tumor clearance. Mol Ther 2012; 20:2076-86; PMID:22828500; http://dx.doi.org/10.1038/mt.2012.137
176. Zhang H, Liu L, Yu D, Kandimalla ER, Sun HB, Agrawal S, et al. An in situ autologous tumor vaccination with combined radiation therapy and TLR9 agonist therapy. PLoS One 2012; 7:e38111; PMID:22666458; http://dx.doi.org/10.1371/journal.pone.0038111
177. Koga-Yamakawa E, Dovedi SJ, Murata M, Matsui H, Leishman AJ, Bell J, et al. Intratracheal and oral administration of SM-276001: a selective TLR7 agonist, leads to antitumor efficacy in primary and metastatic models of cancer. Int J Cancer 2013; 132:580-90; PMID:22733292; http://dx.doi.org/10.1002/ijc.27691
178. Hong X, Dong T, Hu J, Yi T, Li W, Zhang Z, et al. Synergistical toll-like receptors activated dendritic cells induce antitumor effects against carcinoembryonic antigen-expressing colon cancer. Int J Colorectal Dis 2013; 28:25-33; PMID:22777000; http://dx.doi.org/10.1007/s00384-012-1530-7
179. Shirota Y, Shirota H, Klinman DM. Intratumoral injection of CpG oligonucleotides induces the differentiation and reduces the immunosuppressive activity of myeloid-derived suppressor cells. J Immunol 2012; 188:1592-9; PMID:22231700; http://dx.doi.org/10.4049/jimmunol.1101304
180. Kauffman EC, Liu H, Schwartz MJ, Scherr DS. Toll-like receptor 7 agonist therapy with imidazoquinoline enhances cancer cell death and increases lymphocytic infiltration and proinflammatory cytokine production in established tumors of a renal cell carcinoma mouse model. J Oncol 2012; 2012:103298; PMID:22481916; http://dx.doi.org/10.1155/2012/103298
181. Sommariva M, de Cesare M, Meini A, Cataldo A, Zaffaroni N, Tagliabue E, et al. High efficacy of CpG-ODN, Cetuximab and Cisplatin combination for very advanced ovarian xenograft tumors. J Transl Med 2013; 11:25; PMID:23360557; http://dx.doi.org/10.1186/1479-5876-11-25
182. Sommariva M, De Cecco L, Tagliabue E, Balsari A. Modulation of DNA repair genes induced by TLR9 agonists: A strategy to eliminate "altered" cells? Oncoimmunology 2012; 1:258-9; PMID:22720263; http://dx.doi.org/10.4161/onci.1.2.18343
183. Estornes Y, Toscano F, Virard F, Jacquemin G, Pierrot A, Vanbervliet B, et al. dsRNA induces apoptosis through an atypical death complex associating TLR3 to caspase-8. Cell Death Differ 2012; 19:1482-94; PMID:22421964; http://dx.doi.org/10.1038/cdd.2012.22
184. Weiss R, Sachet M, Zinngrebe J, Aschacher T, Krainer M, Hegedus B, et al. IL-24 sensitizes tumor cells to TLR3-mediated apoptosis. Cell Death Differ 2013; 20:823-33; PMID:23449395; http://dx.doi.org/10.1038/cdd.2013.15
185. Chen L, Xu YY, Zhou JM, Wu YY, e Q, Zhu YY. TLR3 dsRNA agonist inhibits growth and invasion of HepG2.2.15 HCC cells. Oncol Rep 2012; 28:200-6; PMID:22552584
186. Guo Z, Chen L, Zhu Y, Zhang Y, He S, Qin J, et al. Double-stranded RNA-induced TLR3 activation inhibits angiogenesis and triggers apoptosis of human hepatocellular carcinoma cells. Oncol Rep 2012; 27:396-402; PMID:22075935
187. Hsiao CC, Kao YH, Huang SC, Chuang JH. Toll-like receptor-4 agonist inhibits motility and invasion of hepatoblastoma HepG2 cells in vitro. Pediatr Blood Cancer 2013; 60:248-53; PMID:22648929; http://dx.doi.org/10.1002/pbc.24211
188. Tano T, Okamoto M, Kan S, Nakashiro K, Shimodaira S, Yamashita N, et al. Growth inhibition and apoptosis by an active component of OK-432, a streptococcal agent, via Toll-like receptor 4 in human head and neck cancer cell lines. Oral Oncol 2012; 48:678-85; PMID:22387210; http://dx.doi.org/10.1016/j.oraloncology.2012.02.005
189. Ahn MY, Kwon SM, Cheong HH, Park JH, Lee J, Min SK, et al. Toll-like receptor 7 agonist, imiquimod, inhibits oral squamous carcinoma cells through apoptosis and necrosis. J Oral Pathol Med 2012; 41:540-6; PMID:22577802
190. Min R, Siyi L, Wenjun Y, Shengwen L, Ow A, Lizheng W, et al. Toll-like receptor-9 agonists increase cyclin D1 expression partly through activation of activator protein-1 in human oral squamous cell carcinoma cells. Cancer Sci 2012; 103:1938-45; PMID:22853846; http://dx.doi.org/10.1111/j.1349-7006.2012.02394.x
191. Hattar K, Savai R, Subtil FS, Wilhelm J, Schmall A, Lang DS, et al. Endotoxin induces proliferation of NSCLC in vitro and in vivo: role of COX-2 and EGFR activation. Cancer Immunol Immunother 2013; 62:309-20; PMID:22923191; http://dx.doi.org/10.1007/s00262-012-1341-2
192. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell 2012; 21:504-16; PMID:22516259; http://dx.doi.org/10.1016/j.ccr.2012.02.007
193. Egunsola AT, Zawislak CL, Akuffo AA, Chalmers SA, Ewer JC, Vail CM, et al. Growth, metastasis, and expression of CCL2 and CCL5 by murine mammary carcinomas are dependent upon Myd88. Cell Immunol 2012; 272:220-9; PMID:22088941; http://dx.doi.org/10.1016/j.cellimm.2011.10.008
194. Wang C, Fei G, Liu Z, Li Q, Xu Z, Ren T. HMGB1 was a pivotal synergistic effecor for CpG oligonucleotide to enhance the progression of human lung cancer cells. Cancer Biol Ther 2012; 13:727-36; PMID:22617774; http://dx.doi.org/10.4161/cbt.20555
195. Wu FH, Yuan Y, Li D, Liao SJ, Yan B, Wei JJ, et al. Extracellular HSPA1A promotes the growth of hepatocarcinoma by augmenting tumor cell proliferation and apoptosis-resistance. Cancer Lett 2012; 317:157-64; PMID:22115967; http://dx.doi.org/10.1016/j.canlet.2011.11.020
196. Liang B, Chen R, Wang T, Cao L, Liu Y, Yin F, et al. Myeloid differentiation factor 88 promotes growth and metastasis of human hepatocellular carcinoma. Clin Cancer Res 2013; 19:2905-16; PMID:23549880; http://dx.doi.org/10.1158/1078-0432.CCR-12-1245
197. Luo Y, Chihara Y, Fujimoto K, Sasahira T, Kuwada M, Fujiwara R, et al. High mobility group box 1 released from necrotic cells enhances regrowth and metastasis of cancer cells that have survived chemotherapy. Eur J Cancer 2013; 49:741-51; PMID:23040637; http://dx.doi.org/10.1016/j.ejca.2012.09.016
198. Mardente S, Mari E, Consorti F, Di Gioia C, Negri R, Etna M, et al. HMGB1 induces the overexpression of miR-222 and miR-221 and increases growth and motility in papillary thyroid cancer cells. Oncol Rep 2012; 28:2285-9; PMID:23023232
199. Tye H, Kennedy CL, Najdovska M, McLeod L, McCormack W, Hughes N, et al. STAT3-driven upregulation of TLR2 promotes gastric tumorigenesis independent of tumor inflammation. Cancer Cell 2012; 22:466-78; PMID:23079657; http://dx.doi.org/10.1016/j.ccr.2012.08.010
200. Rakhesh M, Cate M, Vijay R, Shrikant A, Shanjana AA. A TLR4-interacting peptide inhibits lipopolysaccharide-stimulated inflammatory responses, migration and invasion of colon cancer SW480 cells. Oncoimmunology 2012; 1:1495-506; PMID:23264896; http://dx.doi.org/10.4161/onci.22089
201. Huang Y, Cai B, Xu M, Qiu Z, Tao Y, Zhang Y, et al. Gene silencing of Toll-like receptor 2 inhibits proliferation of human liver cancer cells and secretion of inflammatory cytokines. PLoS One 2012; 7:e38890; PMID:22815694; http://dx.doi.org/10.1371/journal.pone.0038890
202. Liao SJ, Zhou YH, Yuan Y, Li D, Wu FH, Wang Q, et al. Triggering of Toll-like receptor 4 on metastatic breast cancer cells promotes αvβ3-mediated adhesion and invasive migration. Breast Cancer Res Treat 2012; 133:853-63; PMID:22042369; http://dx.doi.org/10.1007/s10549-011-1844-0
203. Jing YY, Han ZP, Sun K, Zhang SS, Hou J, Liu Y, et al. Toll-like receptor 4 signaling promotes epithelial-mesenchymal transition in human hepatocellular carcinoma induced by lipopolysaccharide. BMC Med 2012; 10:98; PMID:22938142; http://dx.doi.org/10.1186/1741-7015-10-98
204. Källberg E, Vogl T, Liberg D, Olsson A, Björk P, Wikström P, et al. S100A9 interaction with TLR4 promotes tumor growth. PLoS One 2012; 7:e34207; PMID:22470535; http://dx.doi.org/10.1371/journal.pone.0034207
205. Leanderson T, Ivars F. S100A9 and tumor growth. Oncoimmunology 2012; 1:1404-5; PMID:23243608; http://dx.doi.org/10.4161/onci.21027
206. Kauppila JH, Karttunen TJ, Saarnio J, Nyberg P, Salo T, Graves DE, et al. Short DNA sequences and bacterial DNA induce esophageal, gastric, and colorectal cancer cell invasion. APMIS 2012; 121:511-22; PMID:23082743
207. O'Leary DP, Bhatt L, Woolley JF, Gough DR, Wang JH, Cotter TG, et al. TLR-4 signalling accelerates colon cancer cell adhesion via NF-κB mediated transcriptional up-regulation of Nox-1. PLoS One 2012; 7:e44176; PMID:23071493; http://dx.doi.org/10.1371/journal.pone.0044176

208. Yan W, Chang Y, Liang X, Cardinal JS, Huang H, Thorne SH, et al. High-mobility group box 1 activates caspase-1 and promotes hepatocellular carcinoma invasiveness and metastases. Hepatology 2012; 55:1863-75; PMID:22234969; http://dx.doi.org/10.1002/hep.25572

209. Zhang Y, Wang Y, Yuan J, Qin W, Liu F, Wang F, et al. Toll-like receptor 4 ligation confers chemoresistance to docetaxel on PC-3 human prostate cancer cells. Cell Biol Toxicol 2012; 28:269-77; PMID:22648782; http://dx.doi.org/10.1007/s10565-012-9221-2

210. Sun Z, Luo Q, Ye D, Chen W, Chen F. Role of toll-like receptor 4 on the immune escape of human oral squamous cell carcinoma and resistance of cisplatin-induced apoptosis. Mol Cancer 2012; 11:33; PMID:22583829; http://dx.doi.org/10.1186/1476-4598-11-33

211. Tang X, Zhu Y. TLR4 signaling promotes immune escape of human colon cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. Oncol Res 2012; 20:15-24; PMID:23035361; http://dx.doi.org/10.3727/096504012X13425470196092

212. Davoodi H, Hashemi SR, Seow HF. Increased NFk-B activity in HCT116 colorectal cancer cell line harboring TLR4 Asp299Gly variant. Iran J Allergy Asthma Immunol 2012; 11:121-32; PMID:22761186

213. Cheng YX, Qi XY, Huang JL, Hu M, Zhou LM, Li BS, et al. Toll-like receptor 4 signaling promotes the immunosuppressive cytokine production of human cervical cancer. Eur J Gynaecol Oncol 2012; 33:291-4; PMID:22873102

214. Riddell JR, Maier P, Sass SN, Moser MT, Foster BA, Gollnick SO. Peroxiredoxin 1 stimulates endothelial cell expression of VEGF via TLR4 dependent activation of HIF-1α. PLoS One 2012; 7:e50394; PMID:23185615; http://dx.doi.org/10.1371/journal.pone.0050394

215. Hansen J, Lindenstrøm T, Lindberg-Levin J, Aagaard C, Andersen P, Agger EM. CAF05: cationic liposomes that incorporate synthetic cord factor and poly(I:C) induce CTL immunity and reduce tumor burden in mice. Cancer Immunol Immunother 2012; 61:893-903; PMID:22095092; http://dx.doi.org/10.1007/s00262-011-1156-6

216. Huang Z, Yang Y, Jiang Y, Shao J, Sun X, Chen J, et al. Anti-tumor immune responses of tumor-associated macrophages via toll-like receptor 4 triggered by cationic polymers. Biomaterials 2013; 34:746-55; PMID:23107297; http://dx.doi.org/10.1016/j.biomaterials.2012.09.062

217. Wang C, Zhuang Y, Zhang Y, Luo Z, Gao N, Li P, et al. Toll-like receptor 3 agonist complexed with cationic liposome augments vaccine-elicited antitumor immunity by enhancing TLR3-IRF3 signaling and type I interferons in dendritic cells. Vaccine 2012; 30:4790-9; PMID:22634298; http://dx.doi.org/10.1016/j.vaccine.2012.05.027

218. Brennan TV, Lin L, Huang X, Cardona DM, Li Z, Dredge K, et al. Heparan sulfate, an endogenous TLR4 agonist, promotes acute GVHD after allogeneic stem cell transplantation. Blood 2012; 120:2899-908; PMID:22760779; http://dx.doi.org/10.1182/blood-2011-07-368720

219. He S, Chu J, Wu LC, Mao H, Peng Y, Alvarez-Breckenridge CA, et al. MicroRNAs activate natural killer cells through toll-like receptor signaling. Blood 2013; In press; PMID:23580661; http://dx.doi.org/10.1182/blood-2012-07-441360

220. Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, et al. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. Proc Natl Acad Sci U S A 2012; 109:E2110-6; PMID:22753494; http://dx.doi.org/10.1073/pnas.1209414109

221. Yamazaki T, Hannani D, Poirier-Colame V, Ladoire S, Locher C, Prada N, et al. Defective immunogenic cell death of HMGB1-deficient cancers: compensatory therapy with synthetic TLR4 agonists. Cell Death Differ 2013; In press.

222. Koizumi S, Masuko K, Wakita D, Tanaka S, Mitamura R, Kato Y, et al. Extracts of Larix Leptolepis effectively augments the generation of tumor antigen-specific cytotoxic T lymphocytes via activation of dendritic cells in TLR-2 and TLR-4-dependent manner. Cell Immunol 2012; 276:153-61; PMID:22677561; http://dx.doi.org/10.1016/j.cellimm.2012.05.002

223. Zhu J, Ghosh A, Coyle EM, Lee J, Hahm ER, Singh SV, et al. Differential effects of phenethyl isothiocyanate and D,L-sulforaphane on TLR3 signaling. J Immunol 2013; 190:4400-7; PMID:23509350; http://dx.doi.org/10.4049/jimmunol.1202093

224. Shi Z, Cai Z, Yu J, Zhang T, Zhao S, Smeds E, et al. Toll-like receptor 11 (TLR11) prevents Salmonella penetration into the murine Peyer patches. J Biol Chem 2012; 287:43417-23; PMID:23135279; http://dx.doi.org/10.1074/jbc.M112.411009

225. Oldenburg M, Krüger A, Ferstl R, Kaufmann A, Nees G, Sigmund A, et al. TLR13 recognizes bacterial 23S rRNA devoid of erythromycin resistance-forming modification. Science 2012; 337:1111-5; PMID:22821982; http://dx.doi.org/10.1126/science.1220363

226. Bomben R, Gobessi S, Dal Bo M, Volinia S, Marconi D, Tissino E, et al. The miR-17:92 family regulates the response to Toll-like receptor 9 triggering of CLL cells with unmutated IGHV genes. Leukemia 2012; 26:1584-93; PMID:22343732; http://dx.doi.org/10.1038/leu.2012.44

227. Li Q, Li X, Guo Z, Xu F, Xia J, Liu Z, et al. MicroRNA-574-5p was pivotal for TLR9 signaling enhanced tumor progression via down-regulating checkpoint suppressor 1 in human lung cancer. PLoS One 2012; 7:e48278; PMID:23133627; http://dx.doi.org/10.1371/journal.pone.0048278

228. Li D, Jia H, Zhang H, Lv M, Liu J, Zhang Y, et al. TLR4 signaling induces the release of microparticles by tumor cells that regulate inflammatory cytokine IL-6 of macrophages via microRNA let-7b. Oncoimmunology 2012; 1:687-93; PMID:22934260; http://dx.doi.org/10.4161/onci.19854

229. Dibra D, Cutrera JJ, Li S. Coordination between TLR9 signaling in macrophages and CD3 signaling in T cells induces robust expression of IL-30. J Immunol 2012; 188:3709-15; PMID:22407920; http://dx.doi.org/10.4049/jimmunol.1100883

230. Balamurugan K, Sharan S, Klarmann KD, Zhang Y, Coppola V, Summers GH, et al. FBXW7α attenuates inflammatory signalling by downregulating C/EBPδ and its target gene Tlr4. Nat Commun 2013; 4:1662; PMID:23575666; http://dx.doi.org/10.1038/ncomms2677

231. Aksoy E, Taboubi S, Torres D, Delbauve S, Hachani A, Whitehead MA, et al. The p110δ isoform of the kinase PI(3)K controls the subcellular compartmentalization of TLR4 signaling and protects from endotoxic shock. Nat Immunol 2012; 13:1045-54; PMID:23023391; http://dx.doi.org/10.1038/ni.2426

232. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 2009; 9:550-62; PMID:19629070; http://dx.doi.org/10.1038/nrc2664

233. Ahmed A, Wang JH, Redmond HP. Silencing of TLR4 increases tumor progression and lung metastasis in a murine model of breast cancer. Ann Surg Oncol 2012; In press; PMID:22890596; http://dx.doi.org/10.1245/s10434-012-2595-9

234. Lafaille FG, Pessach IM, Zhang SY, Ciancanelli MJ, Herman M, Abhyankar A, et al. Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells. Nature 2012; 491:769-73; PMID:23103873

235. Bernard JJ, Cowing-Zitron C, Nakatsuji T, Muehleisen B, Muto J, Borkowski AW, et al. Ultraviolet radiation damages self noncoding RNA and is detected by TLR3. Nat Med 2012; In press; PMID:22772463; http://dx.doi.org/10.1038/nm.2861

236. Luger R, Valookaran S, Knapp N, Vizzardelli C, Dohnal AM, Felzmann T. Toll-like receptor 4 engagement drives differentiation of human and murine dendritic cells from a pro- into an anti-inflammatory mode. PLoS One 2013; 8:e54879; PMID:23408948; http://dx.doi.org/10.1371/journal.pone.0054879

237. Fujimura T, Kambayashi Y, Aiba S. Crosstalk between regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs) during melanoma growth. Oncoimmunology 2012; 1:1433-4; PMID:23243619; http://dx.doi.org/10.4161/onci.21176

238. Schreiber TH, Wolf D, Bodero M, Podack E. Tumor antigen specific iTreg accumulate in the tumor microenvironment and suppress therapeutic vaccination. Oncoimmunology 2012; 1:642-8; PMID:22934256; http://dx.doi.org/10.4161/onci.20298

239. Weiss VL, Lee TH, Jaffee EM, Armstrong TD. Targeting the right regulatory T-cell population for tumor immunotherapy. Oncoimmunology 2012; 1:1191-3; PMID:23170276; http://dx.doi.org/10.4161/onci.20664

240. Karbach J, Neumann A, Wahle C, Brand K, Gnjatic S, Jäger E. Therapeutic administration of a synthetic CpG oligodeoxynucleotide triggers formation of anti-CpG antibodies. Cancer Res 2012; 72:4304-10; PMID:22738916; http://dx.doi.org/10.1158/0008-5472.CAN-12-0257

241. Lin H, Yan J, Wang Z, Hua F, Yu J, Sun W, et al. Loss of immunity-supported senescence enhances susceptibility to hepatocellular carcinogenesis and progression in Toll-like receptor 2-deficient mice. Hepatology 2013; 57:171-82; PMID:22859216; http://dx.doi.org/10.1002/hep.25991

242. Yevsa T, Kang TW, Zender L. Immune surveillance of pre-cancerous senescent hepatocytes limits hepatocellular carcinoma development. Oncoimmunology 2012; 1:398-9; PMID:22737629; http://dx.doi.org/10.4161/onci.19128

243. Ochi A, Graffeo CS, Zambirinis CP, Rehman A, Hackman M, Fallon N, et al. Toll-like receptor 7 regulates pancreatic carcinogenesis in mice and humans. J Clin Invest 2012; 122:4118-29; PMID:23023703; http://dx.doi.org/10.1172/JCI63606

244. Ochi A, Nguyen AH, Bedrosian AS, Mushlin HM, Zarbakhsh S, Barilla R, et al. MyD88 inhibition amplifies dendritic cell capacity to promote pancreatic carcinogenesis via Th2 cells. J Exp Med 2012; 209:1671-87; PMID:22908323; http://dx.doi.org/10.1084/jem.20111706

245. Zambirinis CP, Miller G. Signaling via MYD88 in the pancreatic tumor microenvironment: A double-edged sword. Oncoimmunology 2013; 2:e22567; PMID:23483804; http://dx.doi.org/10.4161/onci.22567

246. Hovden AO, Karlsen M, Jonsson R, Appel S. The bacterial preparation OK432 induces IL-12p70 secretion in human dendritic cells in a TLR3 dependent manner. PLoS One 2012; 7:e31217; PMID:22363584; http://dx.doi.org/10.1371/journal.pone.0031217

247. Walter A, Schäfer M, Cecconi V, Matter C, Urosevic-Maiwald M, Belloni B, et al. Aldara activates TLR7-independent immune defence. Nat Commun 2013; 4:1560; PMID:23463003; http://dx.doi.org/10.1038/ncomms2566

248. Zitvogel L, Kepp O, Galluzzi L, Kroemer G. Inflammasomes in carcinogenesis and anticancer immune responses. Nat Immunol 2012; 13:343-51; PMID:22430787; http://dx.doi.org/10.1038/ni.2224

249. Margolin K. Ipilimumab in a Phase II trial of melanoma patients with brain metastases. Oncoimmunology 2012; 1:1197-9; PMID:23170278; http://dx.doi.org/10.4161/onci.20687

250. Zitvogel L, Kroemer G. Targeting PD-1/PD-L1 interactions for cancer immunotherapy. Oncoimmunology 2012; 1:1223-5; PMID:23243584; http://dx.doi.org/10.4161/onci.21335

251. Madan RA, Heery CR, Gulley JL. Combination of vaccine and immune checkpoint inhibitor is safe with encouraging clinical activity. Oncoimmunology 2012; 1:1167-8; PMID:23170267; http://dx.doi.org/10.4161/onci.20591
252. Srivastava MK, Dubinett S, Sharma S. Targeting MDSCs enhance therapeutic vaccination responses against lung cancer. Oncoimmunology 2012; 1:1650-1; PMID:23264925; http://dx.doi.org/10.4161/onci.21970
253. Djeu J, Wei S. Chemoimmunomodulation of MDSCs as a novel strategy for cancer therapy. Oncoimmunology 2012; 1:121-2; PMID:22720231; http://dx.doi.org/10.4161/onci.1.1.18074
254. Abrams SI, Waight JD. Identification of a G-CSF-Granulocytic MDSC axis that promotes tumor progression. Oncoimmunology 2012; 1:550-1; PMID:22754783; http://dx.doi.org/10.4161/onci.19334
255. Ghansah T. A novel strategy for modulation of MDSC to enhance cancer immunotherapy. Oncoimmunology 2012; 1:984-5; PMID:23162780; http://dx.doi.org/10.4161/onci.20201
256. Stathopoulos A, Pretto C, Devillers L, Pierre D, Hofman FM, Kruse C, et al. Development of immune memory to glial brain tumors after tumor regression induced by immunotherapeutic Toll-like receptor 7/8 activation. Oncoimmunology 2012; 1:298-305; PMID:22737605; http://dx.doi.org/10.4161/onci.19068
257. Lainey E, Wolfromm A, Marie N, Enot D, Scoazec M, Bouteloup C, et al. Azacytidine and erlotinib exert synergistic effects against acute myeloid leukemia. Oncogene 2012; In press; PMID:23085751; http://dx.doi.org/10.1038/onc.2012.469
258. Pinto A, Rega A, Crother TR, Sorrentino R. Plasmacytoid dendritic cells and their therapeutic activity in cancer. Oncoimmunology 2012; 1:726-34; PMID:22934264; http://dx.doi.org/10.4161/onci.20171
259. Garaude J, Blander JM. "Flagellated" cancer cells propel anti-tumor immunity. Oncoimmunology 2012; 1:940-2; PMID:23162764; http://dx.doi.org/10.4161/onci.20305
260. Burdelya LG, Brackett CM, Kojouharov B, Gitlin II, Leonova KI, Gleiberman AS, et al. Central role of liver in anticancer and radioprotective activities of Toll-like receptor 5 agonist. Proc Natl Acad Sci U S A 2013; 110:E1857-66; PMID:23630282; http://dx.doi.org/10.1073/pnas.1222805110
261. Burdelya LG, Gleiberman AS, Toshkov I, Aygun-Sunar S, Bapardekar M, Manderscheid-Kern P, et al. Toll-like receptor 5 agonist protects mice from dermatitis and oral mucositis caused by local radiation: implications for head-and-neck cancer radiotherapy. Int J Radiat Oncol Biol Phys 2012; 83:228-34; PMID:22000579; http://dx.doi.org/10.1016/j.ijrobp.2011.05.055
262. Burdelya LG, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, et al. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. Science 2008; 320:226-30; PMID:18403709; http://dx.doi.org/10.1126/science.1154986
263. Krieg AM. Therapeutic potential of Toll-like receptor 9 activation. Nat Rev Drug Discov 2006; 5:471-84; PMID:16763660; http://dx.doi.org/10.1038/nrd2059
264. Shirota H, Klinman DM. Effect of CpG ODN on monocytic myeloid derived suppressor cells. Oncoimmunology 2012; 1:780-2; PMID:22934281; http://dx.doi.org/10.4161/onci.19731
265. Hyde MA, Hadley ML, Tristani-Firouzi P, Goldgar D, Bowen GM. A randomized trial of the off-label use of imiquimod, 5%, cream with vs. without tazarotene, 0.1%, gel for the treatment of lentigo maligna, followed by conservative staged excisions. Arch Dermatol 2012; 148:592-6; PMID:22431716; http://dx.doi.org/10.1001/archdermatol.2012.270
266. Spencer DM. Activation of antigen-exposed iMC-DCs at the "right place" and "right time" promotes potent anti-tumor immunity. Oncoimmunology 2012; 1:362-3; PMID:22737615; http://dx.doi.org/10.4161/onci.18482
267. de Vries IJ, Tel J, Benitez-Ribas D, Torensma R, Figdor CG. Prophylactic vaccines mimic synthetic CpG oligonucleotides in their ability to modulate immune responses. Mol Immunol 2011; 48:810-7; PMID:21257206; http://dx.doi.org/10.1016/j.molimm.2010.12.022
268. Schreibelt G, Benitez-Ribas D, Schuurhuis D, Lambeck AJ, van Hout-Kuijer M, Schaft N, et al. Commonly used prophylactic vaccines as an alternative for synthetically produced TLR ligands to mature monocyte-derived dendritic cells. Blood 2010; 116:564-74; PMID:20424184; http://dx.doi.org/10.1182/blood-2009-11-251884