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Clinical evaluation of CpG oligonucleotides as adjuvants for vaccines targeting infectious diseases and cancer

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Abstract

Synthetic oligonucleotides (ODN) that express unmethylated “CpG motifs” trigger cells that express Toll-like receptor 9. In humans this includes plasmacytoid dendritic cells and B cells. CpG ODN induce an innate immune response characterized by the production of Th1 and pro-inflammatory cytokines. Their utility as vaccine adjuvants was evaluated in a number of clinical trials. Results indicate that CpG ODN improve antigen presentation and the generation of vaccine-specific cellular and humoral responses. This work provides an up-to-date overview of the utility of CpG ODN as adjuvants for vaccines targeting infectious agents and cancer.

Keywords

CpG oligonucleotide; Adjuvant; Infection; Cancer; Toll-like receptor

1. Introduction

Vaccines are highly effective public health interventions, having saved millions of lives by preventing infectious diseases. The efficacy of a vaccine is determined by the magnitude, duration and quality of the immune response it induces. Depending upon the disease, both the innate and adaptive arms of the immune system may contribute to protection. The innate immune response involves the activation of multiple cell types, including dendritic cells, macrophages, monocytes, neutrophils, basophils, eosinophils, lymphocytes and/or NK cells. The innate immune system provides a rapid response to pathogens, initiates pathogen clearance, and helps in the healing of damaged tissue. Cells of the adaptive immune system include primarily B and T lymphocytes. Adaptive immune responses develop more slowly but are highly specific and therefore critical for providing sterilizing immunity and long-term memory.

Successful vaccination requires a complex series of immune interactions. Professional APCs (dendritic cells and macrophages) as well as certain other immune cells (e.g. B lymphocytes)

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Conflict of interest

Dr. Klinman and members of his lab have patents related to the use of CpG ODN. All rights to such patents have been assigned to the Federal Government.

take up the vaccine Ag. The Ag is then digested and fragments presented to T lymphocytes via cell–cell interactions that require additional surface receptors. Ag-activated CD4 T cells provide help to Ag-specific B cells, supporting their proliferation, switch recombination and somatic hypermutation, resulting in the production of high-affinity Ab (an outcome measured in many clinical trials) [1–3]. CD8 T cells are also stimulated to proliferate and mature into cytotoxic effectors. These are typically measured by their production of Th1 cytokines, such as IFN- γ . Over the course of the vaccine-elicited response, Ag-specific memory B and T lymphocytes arise that persist long-term and provide protection from subsequent challenge [4–7].

Toll-like receptors (TLR) belong to the family of pathogen recognition receptors that are triggered by pathogen-associated molecular patterns expressed by bacteria, viruses, fungi and protozoa. The basic structure of these receptors include an extracellular leucine-rich repeat region, a transmembrane domain and a cytosolic Toll/Interleukin-1 receptor domain. TLR stimulation contributes to the induction and maintenance of innate and adaptive immune pathways as well as memory function [3]. Eleven human and 13 mouse TLRs have been identified. These TLRs have been categorized based on their ligand specificity, signal transduction pathways, cellular expression profiles and subcellular localization. While human TLRs 1, 2, 4, 5 and 6 are localized on the cell membrane, TLRs 3, 7, 8, 9 and 10 are embedded in intracellular vesicles [8,9], although TLR9 was found on the cellular membrane of human CD19+ B cells [10].

TLRs are expressed primarily on immune cells although other cells are known to contribute to host protection, such as barrier epithelial cells, can also express these receptors. Once triggered by pathogen, these cells produce pro-inflammatory cytokines and chemokines that support the recruitment of additional inflammatory mediators, type I interferon and antimicrobial peptides [11]. TLRs are also expressed on malignant cells and may play a role in oncogenesis and tumor progression by influencing the tumor microenvironment [9,12–14].

2. Toll-like receptor 9

TLR9 recognizes the unmethylated CpG motifs present at high frequency in bacterial but rare in mammalian DNA. The TLR9 receptor is localized to in the endoplasmatic reticulum, late endosomal and lysosomal compartments of the intracellular milieu. Thus, internalization of pathogen-derived DNA is required for TLR9 triggering, an outcome that results from either intracellular infection or uptake of bacterial/viral particles by immune cells [15]. Once stimulated, TLR9 initiates a response biased towards pro-inflammatory/Th1 biased immunity [16].

TLR9 molecules differ between species, with the structure of human versus mouse TLR9 varying by 24% [17]. There is also variation between species in terms of which cell types express TLR9. For example, the TLR9 receptor is present in rodent but not in primate macrophages and myeloid dendritic cells (mDC). In humans, TLR9 is expressed primarily by plasmacytoid CD (pDC) and B cells [18–21]. Reflecting their utility as vaccine adjuvants, B lymphocytes exposed to TLR9 agonists become more susceptible to activation

by Ag [22–24] while TLR9 stimulated pDC produce type I interferons and more efficiently present Ag to T cells [25–27]. Non-human primates (NHP) also respond to the same CpG motifs as humans. Thus, results from NHP are better predictors of human immunological responses to CpG ODN than rodents.

3. TLR9-mediated signaling cascade

The binding of CpG DNA to TLR9 induces proteolytic cleavage of the receptor [28,29]. After exiting the ER, the TLR9 ectodomain is cleaved by asparagine endopeptidase and/or cathepsins [30,31]. This truncated (rather than full length) form of TLR9 recruits myeloid differentiation factor 88 (MyD88) such that if proteolysis of the receptor is prevented, it becomes non-functional. The requirement for ectodomain cleavage provides an ancillary mechanism to restrict receptor activation to endolysosomal compartments and further prevents TLR9 from responding to self DNA. The signaling pathway triggered by the interaction of CpG DNA with TLR9 proceeds through the recruitment of MyD88, IL-1R-associated kinase (IRAK) and tumor necrosis factor receptor-associated factor 6 (TRAF6), and subsequently involves the activation of several mitogen-activated kinases (MAPK) and transcription factors (such as NF- κ B and AP-1) culminating in the transcription of proinflammatory chemokines and cytokines [32]. While TLR9 expressing cells recognize unmethylated CpG motifs, other molecules may support the signal transduction. For example DEC-205, a multi-lectin receptor that can bind CpG motifs and has been shown to facilitate the uptake of CpG ODN by DC and B cells [33].

4. CpG ODN

Bacterial DNA is the native ligand for TLR9 and synthetic oligonucleotides (ODN) that mimic the structure of bacterial DNA duplicate this activity. In humans, four distinct classes of CpG ODN have been identified based on differences in structure and the nature of the immune response they induce. Although each class contains at least one motif composed of a central unmethylated CG dinucleotide plus flanking regions, they differ in structure and immunological activity.

K-type ODNs (also referred to as B-type) contain from 1 to 5 CpG motifs typically on a phosphorothioate backbone. This backbone enhances resistance to nuclease digestion and substantially prolongs *in vivo* half-life (30–60 min compared with 5–10 min for phosphodiester) [34]. K-type ODNs trigger pDC to differentiate and produce TNF- α and stimulate B cells to proliferate and secrete IgM [35,36]. Extensive clinical trials involving K-type ODN have been conducted (as reviewed below).

D-type ODNs (also referred to as A-type) have a phosphodiester core flanked by phosphorothioate terminal nucleotides. They carry a single CpG motif flanked by palindromic sequences that enables the formation of a stem-loop structure. D-type ODN also has poly G motifs at the 3' and 5' ends that facilitate concatamer formation. D-type ODN trigger pDC to mature and secrete IFN- α but have no effect on B cells [35,37]. The distinct activities of K- versus D-type ODNs are largely due to differences in the retention times of CpG/TLR9 complexes in the endosomes of pDC [38,39]. Whereas K-type ODNs are rapidly transported through early endosomes into late endosomes, D-type ODNs are retained for

longer periods in the early endosome. It is in the early endosomes that D-type ODNs interact with MyD88/IRF-7 complexes, triggering a signaling cascade that supports IFN- α production [39]. D-type ODN tend to form complex multimers in solution due to interactions between their poly-G tails, and thus has posed a barrier to their use in clinical trials. However D-type ODN were recently packaged into stable virus like particles (VLP) and used as adjuvants in preclinical and clinical studies [40–42].

C-type ODNs resemble K-type in being composed entirely of phosphorothioate nucleotides but resemble D-type in containing palindromic CpG motifs that can form stem loop structures or dimers. This class of ODN stimulates B cells to secrete IL-6 and pDC to produce IFN- α . C-type ODNs have activity in both early and late endosomes, and thus express properties in common with both K- and D-type ODNs [43,44]. Phosphodiester linkages can be introduced into the CG dinucleotides (referred to as semi-soft C), a modification reported to further enhance the activity of C-type ODN. P-Class CpG ODN contain double palindromes that can form hairpins at their GC-rich 3' ends as well as concatamerize due to the presence of the 5' palindromes. These highly ordered structures are credited with inducing the strongest type I IFN production of any class of CpG ODN [45,46].

Stimulation via TLR9 results in the rapid activation of the innate immune system that in turn supports the induction of an adaptive immune response. This series of effects provides a mechanism by which CpG ODN might be harnessed as a vaccine adjuvant. Extensive animal testing showed that CpG ODN could support the induction of Ag-specific immunity against co-administered peptides and vaccines [47,48]. Below, we review the literature by focusing on clinical studies that examined the utility of CpG ODN as an adjuvant for vaccines targeting infectious diseases (Table 1) and cancer (Table 2). Most clinical trials evaluated the activity of CpG ODN adjuvanted vaccines by monitoring specific responses such as Ab titers, cytokine levels, cell proliferation and changes in the frequency of CTL, NK cells, CD8 and CD4 T cells. For vaccines targeting infectious agents, the efficacy of adding CpG ODN was typically examined by comparison to a control group that received vaccine only. In cancer vaccine trials, this critical control group was often missing which complicates interpretation of the reported results.

5. Clinical trials that include CpG ODN in vaccines targeting infectious diseases

5.1. Anthrax

Anthrax is caused by the toxin-producing gram positive bacterium *Bacillus Anthracis*. The spores of this pathogen are highly resistant to environmental degradation (desiccation, heat, UV light) and many disinfectant solutions [49]. Protection against anthrax is provided by Abs targeting the 'protective antigen' (PA) component of the toxin.

The currently licensed human vaccine, Anthrax Vaccine Adsorbed (AVA) is administered as 6 subcutaneous (SC) injections over 18 months followed by annual boosters. A range of adverse events (AEs), including injection site pain, joint pain, flu-like symptoms and even pneumonia was observed as the number of vaccinations increases [50,51]. In an effort to

reduce AEs and accelerate the induction and duration of protective immunity, the effect of adding K-type CpG ODN to AVA was examined. In multiple animal models combining CpG ODN with AVA improved immunogenicity and accelerated the generation of PA-specific Abs [48,52]. Such findings supported the initiation of several clinical trials which consistently showed that adding CpG ODN to AVA increased serum IgG anti-PA titers by 6–8 fold ($p < 0.001$) while reducing the time needed to induce a protective titer by nearly 3 weeks ($p < 0.001$) [53]. A similar increase and acceleration in toxin neutralizing Ab levels was also observed. More recently, a study evaluating two doses of AVA delivered IM found that adding CpG 7909 again significantly improved Ab titers and accelerated the induction of immunity [54].

5.2. Hepatitis B

Hepatitis B (HBV) is a hepadnavirus that predominantly infects hepatocytes. The resulting infection can lead to acute or chronic hepatitis, the latter being associated with long-term complications including cirrhosis and hepatocellular carcinoma. Vaccination has reduced the incidence and complications of HBV infection worldwide in the past 3 decades. Yet HBV remains a major public health issue among individuals who respond poorly to the licensed vaccines [55].

Efforts to produce a more immunogenic HBV vaccine have focused on use of HBsAg which is expressed on the surface of HBV and is released into the serum following infection. It acts as the major antigenic component of current HBV vaccines. Pre-clinical studies showed that CpG 2216 and CpG 2395 increased the recognition of HBV epitopes [56] and triggered a 2–4 fold increase in the production of IFN- γ and IL-4 by PBMC from patients with chronic HBV infection [57]. Rodents and primates treated with HBsAg adjuvanted with CpG 2006 had higher response rates (a dose-dependent effect) and produced significantly higher levels of IgG2A and IgG2B anti-HBsAg Ab (2–10 fold; $p = 0.005$) when compared to vaccine alone [58–60]. Similar improvements were observed when CpG 7909 and ISS 1018 were evaluated in clinical trials: normal volunteers generated significantly 3–10 fold higher anti-HBsAg Ab titers when immunized with adjuvanted vaccine vs. vaccine alone [61–63]. ODN 7909 at a dose of 0.5 mg was the most effective, inducing seroprotective titers in all subjects that were maintained through week 24 (vs. 55% with vaccine alone, $p = 0.0003$) [61]. In a phase I dose escalation study of healthy adults, 1–3 mg of ISS 1018 (K/B-type ODN) generated higher anti-HBsAg in a greater fraction of participants after the first and second immunization than vaccine alone ($p = 0.001$) [62]. A recent phase III trial compared the effect of recombinant HBsAg plus 3 mg of ISS 1018 to the Engerix-B[®] vaccine in healthy individuals. CpG-HBsAg co-vaccination yielded a higher percentage of seroprotected participants at 4 (23% vs. 3%), 8 (88% vs. 26%), 24 (98% vs. 32%) and 28 weeks (97% vs. 81%) and generated peak Ab titers after only 2 vaccinations vs. 3 vaccinations for the Engerix-B[®] group [64]. The use of HBsAg plus ISS 1018 vs. Engerix-B[®] was also evaluated in adults 40–70 years of age. As above, seroprotective titers were achieved in more participants vaccinated with ISS-HBsAg at 4 weeks (96% vs. 24%) and 28 weeks (100% vs. 73%; $p < 0.0001$). Moreover, titers of anti-HBsAg Ab exceeding 100 mIU/mL were induced in 97% vs. 58% at week 28 and were maintained in 90% vs. 43% through week 50

compared to Engerix-B® ($p < 0.0001$) [65]. These trials clearly establish the improved immunogenicity of a CpG ODN adjuvanted vaccine.

5.3. Malaria

A number of clinical trials evaluated the use of CpG ODN as adjuvants for vaccines against malaria [66,67]. In the blood stage of malaria infection, parasite-specific *Plasmodium falciparum* (PF) Abs play a critical role in controlling parasite numbers [67,68]. Natural infection elicits memory B cells and long lived plasma cell response that provided malaria-specific Abs and reduced disease burden [69,70]. An object of vaccination is to reproduce the natural protection. Preclinical studies showed that immunization with a vaccine containing the apical membrane antigen 1 (AMA1) of malaria, a surface protein expressed during the asexual blood-stage of PF, induced Abs and that protected against homologous parasite challenge in rodents [71]. Yet studies in Aotus monkeys showed that *in vivo* protection required considerably higher anti-AMA 1 Ab titers than were achieved by vaccination with the CpG ODN adjuvanted vaccine [72].

In human clinical trials involving malaria-exposed adults, supplementing an AMA1-C1 vaccine formulated in Alhydrogel® with CpG 7909 improved the resultant anti-AMA-1 Ab response by 2–3 fold ($p = 0.013$) [73,74]. An even greater improvement in AMA1-specific Ab titers (up to 14-fold) was observed when CpG 7909 was added to this vaccine and administered to malaria-naïve individuals [75–77]. A positive correlation was observed between the frequency of vaccine-specific memory B cells and Ab levels after re-immunization (AMA1-C1: $r = 0.80$; MSP42: $r = 0.86$). Serum from subjects vaccinated with the CpG 7909 adjuvanted vaccine provided 3–4 fold higher inhibition of PF growth *in vitro* than serum from individuals vaccinated with AMA 1 alone ($p < 0.0001$) [76,78]. Unfortunately, the Ab titers achieved in subjects vaccinated with the CpG 7909 adjuvanted vaccine were insufficient to protect against malaria infection or even reduce the multiplication rate of PF rate *in vivo* ($p = 0.7$) [78]. This data elicit special difficulties in generating vaccine strategy to provide immunity to malaria as an intracellular parasite.

5.4. Influenza

The addition of CpG ODN to licensed influenza vaccines was found to improve immunogenicity in multiple animal models [79–81]. For example, adding CpG ODN to the human influenza vaccine Fluviral® (including antigens A/Sydney, A/Beijing and B/Harbin) induced higher virus-specific Ab titers resulting in better protection in ferrets as manifest by a 20% reduction in viral load when compared to Fluviral® alone [81].

A randomized, blinded, phase Ib trial was conducted in which subjects were vaccinated with either 1/10th or a full-dose of Fluarix® alone or co-administered with 1 mg of the CpG 7909 ($n = 15$ per group). The inclusion of CpG 7909 did not improve hemagglutination inhibition (HI) Ab titers or total IgG levels in the serum ($p > 0.05$). However, recipients of the 1/10th dose of Fluarix® vaccine combined with CpG 7909 had 4–7 fold higher IFN- γ producing PBMCs after stimulation with the antigens A/Beijing/262/95 ($p = 0.048$) and B/Harbin/7/94 ($p = 0.0057$) on day 28 compared to low dose vaccine recipients alone, which was nearly

equivalent to a full dose of Fluarix® in the absence of adjuvant. These results are consistent with a dose-sparing effect of CpG ODN in humans [82].

6. CpG ODN as an adjuvant in HIV-infected individuals

Chronic HIV infection is characterized by latent infection and impairment of CD4 T cells and the cytolytic activity of CD8 T cells. This contributes to a diminution in the immunogenicity of standard vaccines, placing HIV patients at higher risk for associated infections and increased mortality [83,84]. CpG ODN were found to boost immunity in mice with depressed immune systems. However their utility in HIV-infected patients was uncertain, since they could potentially increase virus replication levels by stimulating the proliferation of latently infected immune cells [85–87] as was observed in HIV patients with opportunistic bacterial infections [88,89].

Clinical trials described above (see Hepatitis B section) established the safety and efficacy of using CpG 7909 or ISS 1018 as adjuvants for a recombinant HBsAg hepatitis vaccine in healthy volunteers. Those findings supported the initiation of clinical trials of HBV vaccines in patients with HIV infection [90–92]. Anti-HBsAg Ab titers in subjects receiving the CpG 7909 adjuvanted vaccines were 8 fold higher after the second vaccination (week 4) and remained high (4–7 fold) at week 48 of follow up ($p < 0.05$). 89% of the CpG 7909 adjuvanted group responded to vaccination and achieved protective titers by week 6, which was 4 weeks earlier than recipients of Enderix-B® alone. Seroprotective titers (>10 mIU/L) were reached by all individuals in the CpG group by week 10 versus 89% in the control group. Also, protective titers persisted in all members of the CpG group at week 48 after vaccination versus 63% of those immunized with vaccine alone ($p = 0.008$). The frequency of seroprotective Ab levels persisted in the CpG 7909 treated group through 54 months of follow up when compared to the non-CpG group ($p = 0.02$). Even 60 months after vaccination 80% of the CpG 7909 vaccinated individuals maintained seroprotective titers compared to 40% in the non-CpG group ($p = 0.004$). It was also reported that helper T cells from individuals receiving the adjuvanted vaccine mounted 2–3 fold higher responds to Ag-specific stimulation *ex vivo* that did vaccine alone controls for up to 48 months ($p = 0.04$). However, there were no changes in total CD4 or CD8 T cell counts in the peripheral blood nor were significant differences in plasma (HIV p24) viremia observed in these HIV patients, all of whom were maintained under HAART throughout the study [90–92].

A phase Ib/IIa trial was conducted in HAART-naïve and HAART-treated HIV-infected patients vaccinated with the pneumococcal Ag PCV7 (Pneumovax®) and PPV-23 (PneumoNovum®) vaccines [93–95]. After 3 vaccinations, there was a significantly higher percentage of responders (seroprotective titers >10 mIU/mL) in the group that received the CpG 7909 adjuvanted vaccines versus the control group (48.8% vs. 25%, $p = 0.02$) [93]. In the same cohort of patients, increased Ab dependent cellular immunity was detected after the second vaccination accompanied by significantly higher PBMC cytokine responses involving IL-1 ($p = 0.0046$), IL-6 ($p = 0.0051$), IFN- α ($p = 0.0047$), MIP- β ($p = 0.0086$), and IL-2R ($p = 0.0062$). PBMC from the CpG 7909 adjuvanted group responded to LPS stimulation by producing IL-1 β ($p = 0.038$) and IFN- α ($p = 0.019$) at significantly higher levels when compared to non-CpG controls [94]. HIV pro-viral DNA levels fell by 12.6% in

the CpG 7909 treated group versus a rise of 6.7% in the placebo group after the 3rd vaccination (at 10 months) ($p = 0.056$). There were no changes in HIV-specific Ab levels or the frequency of total or CD4 T cells after immunization [95].

7. Clinical trials that include CpG ODN in vaccines targeting cancer

7.1. Use of CpG ODN as stand-alone anti-tumor agents

CpG ODN were evaluated as stand alone anti-tumor agents in several clinical trials. While distinct from their use as vaccine adjuvants, results from those trials are described below as they provide insights into the immunostimulatory activity of CpG ODN. Based on the observation that CpG ODN can stimulate an innate immune response capable of decreasing tumor burden in animal models, CpG 7909 alone at doses up to 0.08 mg/kg SQ and 0.32 mg/kg IV was tested in humans. Evidence of *in vivo* activity was provided by elevations in the concentration of serum IP-10, IFN- α , MIP-1 α and IL-12p40 levels in the serum with increasing dosage of CpG ODN [96]. Phase I clinical trials of healthy volunteers showed that CpG ODN were generally well tolerated without significant toxicity. In most of these trials CpG ODN was administered SQ or IV. The route of administration impacted the nature/strength of the resultant immune response and the occurrence of adverse events. For example, increases in serum cytokine levels and injection site reactions were more common when CpG ODN was administered SQ versus IV [96].

The effect of intra-tumoral administration of CpG ODN was also investigated. A number of preclinical animal studies showed that intra-tumoral injections of CpG ODN elicited a stronger protective response than did systemic delivery of the same agent [97–99]. The mechanism underlying this finding was linked to the ability of TLR-activated cells in the tumor microenvironment to (i) better recognize and present tumor Ag and (ii) convert that tumor milieu from immunosuppressive to immunostimulatory. Intra-tumoral delivery induced stronger long-term immunity and enhanced tumor rejection when compared to systemic administration of CpG ODN in mice. Encouraged by these preclinical results, phase I and II clinical trials in patients with glioblastoma were initiated. Unfortunately, overall results were disappointing as no significant clinical improvement was observed. The continuous intra-tumoral delivery of CpG ODN (4 mg/h) resulted in a modest but not statistically significant improvement in median survival time (6–7 vs. 5 months). After 6 months, 19% of these subjects experienced progression free survival (PFS) versus 15–20% among patients receiving standard radiotherapy and chemotherapy [100,101]. Survival of CpG treated subjects after 1 year was 24%, which is superior to that of historic controls (15%). Due the absence of a concurrent control group, care must be taken not to over-interpret this finding [100,101]. Yet the generally poor prognosis of patients with glioblastoma raises hopes when even a modest prolongation in survival is achieved. Thus, the value of local CpG ODN administration awaits the conduct of a randomized control study.

7.2. Use of CpG ODN as tumor vaccine adjuvants

CpG ODN as stand-alone therapy was generally safe but induced immune responses of insufficient magnitude to clear established tumors. Peptide based vaccines by themselves

generally failed to elicit strong immune responses. Investigation studies of peptide vaccines included against Melan-A/Mart-1, gp100, EGF, Mage-A10, NY-ESO-1, IDM-2101, MAGE-A3 and Wilms' Tumor nuclear protein 1 (WT-1) [102–111]. Thus, a number of clinical trials examined the utility of adding adjuvants such as CpG ODN to peptide-based vaccines targeting various tumor associated antigens (TAAs). Of note, many of these studies simultaneously evaluated other immunostimulatory agents such as Montanide® ISA-51, granulocyte-macrophage colony stimulating factor (GM-CSF) and Incomplete Freund's adjuvant (IFA) in combination with CpG ODN. Although several different CpG ODN were included in these clinical trials, most used the K-type ODN CpG 7909 (also referred as PF 3512676). The early phase I trials focused on determining whether CpG ODN were safe and could improve the immunogenicity of co-administered vaccines. Few conclusions concerning clinical efficacy could be reached due to low patient numbers and lack of relevant controls and randomization. As the results of earlier studies were previously reviewed [112–114], this work will focus on more recent clinical trials in which CpG ODN was used as an adjuvant for peptide vaccines (excluding chemo-and/or radiotherapy, Table 2).

Patients with malignant melanoma were included in several clinical studies of CpG ODN adjuvanted vaccines (Table 2). A prospective Phase I trial involved 24 patients with stage II–IV metastatic melanoma who were vaccinated monthly with Melan-A/MART-1 peptide, Montanide® ISA-51 and CpG 7909 SQ. 10 fold more Melan-A/MART-1 specific T cells were generated by patients immunized with the vaccine containing CpG 7909 vs. the same vaccine lacking CpG ODN. When T cells from CpG 7909 co-vaccinated subjects were re-stimulated *in vitro* with a vaccine derived peptide, their cytokine production of IFN- α , TNF- α , IL-2 and CD107a were increased 2–10 fold ($p < 0.01$) when compared to the non CpG-group [115]. In another clinical trial, 22 patients with stage III–IV melanoma were vaccinated SQ up to 13 times with a combination of MART-1, gp100 and tyrosinase peptides emulsified in Montanide® ISA-51 with CpG 7909 (600 μ g) and GM-CSF. Over 2–7 months of follow up, 10% of these patients had partial remission (PR) and 38% stable disease (SD). Half of the patients with SD developed IFN- γ secreting T cells that recognized the MART-1 Ag post vaccination. The median PFS time in this study was 1.9 months and the median overall survival (OS) was 13.4 months. These outcomes were not superior to the therapeutic alternatives commonly used in patients with stage IV and recurrent melanoma [116,117].

Several studies were published investigating the role of D/Atype CpG ODN incorporated in VLP [41,42]. MelQbG10 consists of the Qb coat protein of bacteriophage coupled to the G10 CpG ODN and the tumor peptides Melan-A/MART-1. A phase I/II clinical trial first tested MelQbG10 in melanoma patients with stage II–IV disease found that this vaccine was safe and induced Melan-A/MART-1 specific T cell responses in 14/22 (66%) patients. There were no differences in response associated with daily versus weekly vaccination strategies or the SQ versus the ID routes of delivery. The inclusion of CpG ODN significantly increased the percentage of central memory T cells compared to patients vaccinated with peptide Melan-A peptide plus IFA alone ($p < 0.05$). Unfortunately the clinical response was poor: even 7 of 22 (32%) patients were free of disease, one patient had PR and one patient

had SD, 2 patients died and the remaining half of the patients had PD [42]. The recent trial tested MelQbG10 in combination with Montanide® ISA-51 and topical 5% Imiquimod cream in 21 patients with stage III/IV malignant melanoma [41]. The primary immune outcome of this study was the appearance of Melan-A-specific T cells. Patients were immunized with MelQbG10 + Montanide® ISA-51 by the SQ, ID or intranodal (IN) routes, with two groups also being treated additionally with topical 5% Imiquimod cream. In total, 76% of the patients responded to MelQbG10 vaccination by generating at least a 2 fold increase in Melan-A specific T cells when compared to pre-vaccination conditions. Significantly higher T cell frequencies were seen in patients vaccinated with MelQbG10 plus Montanide® ISA-51 versus MelQbG10 alone ($p = 0.003$). The group vaccinated SQ generated ≈ 2 fold higher numbers of Ag-specific T cells than those vaccinated IN ($p = 0.001$). Despite these promising immunological improvements, there were no significant differences between groups regarding clinical outcome, with their cancer progressing in $\approx$ half of all patients in each treatment arm. SD was observed in a quarter of the patients in all treatment groups [41].

A phase I study examined the safety, immunogenicity and clinical effect of a vaccine targeting WT-1 peptide in 28 patients [118]. The first group of patients was vaccinated with WT-1 peptide plus Montanide ISA-51 ID. 80% of these individuals failed to complete the 9 planned vaccinations due to disease progression. A second group received the same vaccine supplemented with GM-CSF and was administered SQ. Disease progressed in 75% of patients in this group. The final set of patients received the vaccine supplemented with CpG 7909. 60% of these patients had SD, a frequency much higher than observed in the other two groups [118]. No data on the statistical significance of this result is provided, but the beneficial trend in outcome warrants further investigation.

7.3. Safety

More than a thousand subjects participated in the clinical trials described in this review. The dose of ODN commonly used in these trials was 1.5–15 $\mu\text{g/kg}$ and the schedule of ODN administration ranged from weekly to monthly. In preclinical studies, much higher doses of CpG ODN (2.5 mg/kg) were administered daily to mice. These led to a number of serious AEs including liver toxicity, enlargement of the spleen and lymph nodes, extramedullary hematopoiesis and systemic inflammation [119,120]. Yet the lower dose and frequency of CpG ODN used in human vaccine trials, combined with differences in the cell types expressing TLR9 in primates versus rodents, yielded a relatively benign toxicity profile.

Flu-like symptoms including fatigue, rigors, myalgia and pyrexia, and injection site reaction were the most commonly described treatment related AEs. These events were primarily grade 1–2 and their frequency tended to increase with CpG ODN dose. The likelihood of an injection site reaction arose significantly as subjects received additional doses of CpG ODN adjuvanted vaccines, but the severity of these reactions remained mild to moderate ($p < 0.05$) [90]. For instance 1 of 24 patients (4%) developed generalized pruritus and required oral antihistamin after the first immunization with AMA1-C1/Alhydrogel® + CpG 7909 [74].

CpG ODN also induced biphasic changes in leukocyte frequency in the peripheral blood when administered SQ. In one study, neutrophil numbers doubled at 12 h and then fell to half of baseline levels at 72 h after each vaccination, in some cases reaching a nadir of 800 cells/ μ l. These changes in the peripheral blood may reflect the movement of immune cells through the blood to the site of CpG ODN vaccination and associated draining lymph nodes [121–124]. Lymphadenopathy was reported in two studies [41,42] and enlarged lymph nodes were detected in 9 of 21 patients (42%) after vaccination with MelQbG10 in combination with Montanide[®] ISA-51 regardless the disease status [41].

Concern was raised early in their development that CpG ODN might activate autoreactive B cells and thus increase the risk of autoimmune disease [125]. This possibility was examined in multiple studies. Most detected no significant change in serum autoAb levels against dsDNA, rheumatoid factor, thyroglobulin or nuclear Abs, nor were symptoms of autoimmunity reported [74,116,126,127]. However, 1 of 56 healthy volunteers (2%) vaccinated with Engerix-B[®] plus 1 mg of CpG 7909 developed moderate anti-dsDNA Abs at week 6 which returned to baseline by week 24 [61]. Elevated ANA and anti-dsDNA Ab levels were found in 8/75 (10%) subjects vaccinated with AMA1-C1/Alhydrogel[®] + CpG ODN but these generally returned to baseline within 6 weeks [76]. Thus it appears that CpG ODN are safe and well tolerated when used as an adjuvants in normal healthy individuals.

More frequent and serious AEs were observed in studies involving HIV-infected individuals and patients with cancer. In many situations it was unclear whether the reported effects were causally linked to vaccination or due to the severe nature of the underlying disease (such as the heart attack observed in one subject in onetrial). Although 3 of 29 (10%) HIV-infected patients vaccinated with Engerix-B[®] plus CpG 7909 developed severe AEs, the nature and timing of these AEs is inconsistent with the vaccine having been their cause. These occurred from 0.5 to 4 months post vaccination [90]. Four of 29 patients in this study developed transient elevations in serum anti-ds DNA autoAb levels although no clinical symptoms of autoimmunity were observed [90]. One case of Sjogren's like syndrome was reported in a single trial of 50 patients (2%) with refractory B cell non-Hodgkin's lymphoma. Symptoms in that case included inflammation of the salivary glands and kerato-conjunctivitis sicca after treatment with rituximab and 4 weekly vaccinations with CpG 7909, findings possibly related to development of a paraneoplastic autoimmune disease [122].

Autoimmune responses were developed more frequently in cancer patients [128,129]. In a small trial of patients with prostate cancer, Ab reactive with CpG ODN were detected in 13% of participants without clinical sequelae [128]. Similarly, anti-dsDNA Abs were detected in 4/8 (50%) melanoma patients after four vaccinations with Melan-A, Montanide[®] ISA-51 and CpG 7909, although no clinical signs of autoimmune disease were observed [129]. When CpG ODN were administered intra-cerebrally to patients with glioblastoma, treatment-related general and partial seizures developed in 10% of patients [101]. In the same study, grade 3–4 hematologic events occurred in 2% of recipients of either 10 or 40 mg of CpG ODN [101]. In overview, concerns remain that CpG ODN may have serious side effects when administered to patients with severe infectious disease or cancer, requiring close monitoring of such individuals.

7.4. Limitations

Several considerations limit the interpretation of results from the clinical trials described above and in Table 2. Most tumor vaccine trials suffer from one or more deficiencies that compromise conclusions concerning clinical efficacy. These included (i) low patient number (leaving most trials underpowered), (ii) short duration (providing insufficient follow up to determine whether therapy altered tumor growth) and (iii) inclusion of patients with different types and severity of cancer (preventing intra-group comparison of outcome).

Efforts to evaluate the effect of vaccination on cellular and humoral immune responses were also compromised. Individual studies monitored different immunologic outcomes (ranging from changes in the frequency to activation state of primary and/or memory T cells and NK cells) reflecting a lack of consensus regarding which (if any) are relevant markers of anti-tumor efficacy. Moreover, while animal studies show that the tumor-infiltrating immune cells can provide a useful metric of vaccine efficacy, clinical trials typically rely on peripheral blood samples to monitor the nature and magnitude of the T and B cell responses elicited (as it is difficult to obtain repeated tumor biopsies from cancer patients). This raises the concern that cells in the blood may not reflect the behavior of the functionally relevant cells infiltrating the tumor [130].

8. Conclusion and perspectives

This review focuses on the outcome of clinical trials in which CpG ODN were combined with vaccines targeting infectious diseases and cancer. Overall findings indicate that CpG ODN improve humoral and cellular immune responses when vaccines are administered to healthy individuals. CpG ODN accelerated the induction of protective Abs and supported the generation of higher and more persistent Ab titers with protein vaccines. This was best demonstrated by the extensive trials involving vaccines against anthrax and HBV [53,54,62]. An additional advantage of using CpG ODN as adjuvant was improved immunity even in subjects with compromised immune systems [90,91,61].

Less definitive conclusions can be drawn concerning cancer vaccines. In most studies, CpG ODN increased the immunogenicity of co-administered peptide vaccines (Table 2). Patients who received the adjuvanted vaccines generated stronger Ag-specific serum Ab, CD8 and CD4 T cell responses when compared to peptide vaccines alone. Anti-tumor immunity also arose more rapidly in patients vaccinated with CpG adjuvanted vaccines. These effects were exemplified in the controlled phase I trial of patients with metastatic melanoma. For example, recipients of the CpG ODN adjuvanted vaccine developed Ag-specific CD8 T cells earlier (at the second vs. fourth vaccination) and with significant higher frequency ($1.15\% \pm 0.93\%$ vs. $0.13\% \pm 0.11\%$, $p < 0.01$) than the non-CpG group [129]. These CD8 T cells were of the effector memory phenotype ($CD45RA^{-}$, $CCR7^{-}$ and expressed elevated levels of granzyme B, perforin, $IFN-\gamma$, and $TNF-\alpha$) [129]. However the magnitude of immune effects varied between trials and there was little reproducibility in the effect of CpG ODN on other immune parameters such as the frequency of NK cells or DC [118,131–133]. Of greater concern, the immune responses observed rarely correlated with clinical outcome. Indeed, evidence is lacking that any of the vaccine/adjuvant formulations examined reproducibly altered the progression of large established tumors.

Given the absence of clinical efficacy, we conclude that the magnitude of the immune response needed to eliminate large established tumors exceeds that required to prevent the proliferation of an infectious pathogen, and that available vaccine/CpG ODN combinations do not achieve such a high levels of immunity. The recent success of vaccines targeting prostate tumors provides guidance on how to best structure future clinical trials examining CpG ODN based adjuvants [134]. They suggest that vaccination should be initiated early in the disease process (rather than waiting for all conventional therapies to fail) and targeting small slow growing tumors rather than patients with large tumor burden. Enrolling patients with the same type of cancer and clinical presentation would also facilitate analysis of the results. Finally, we recommend that the raw data from all clinical trials be made freely available, a step that would facilitate the meta-analysis of such data and aid in the rational design of future clinical trials.

Abbreviations

Ab	antibody
AE	adverse event
Ag	antigen
APC	Ag presenting cell
CTL	cytotoxic T cell
CR	complete response
DC	dendritic cell
GIA	growth inhibition assay
GMA	geometric mean Ab titer
HI	hemagglutinin inhibition
ID	intradermal
IM	intramuscular
IN	intranodal
IV	intravenous
ISS	immunostimulatory DNA sequence
NA	not available
NK	natural killer cell
NSCLC	non-small cell lung cancer
OS	overall survival
PBMC	peripheral blood mononuclear cells
PD	progressive disease
PF	Plasmodium falciparum

PFS	progression free survival
PMR	parasite multiplication rate
PR	partial response
SD	stable disease
SQ	subcutaneous
TNA	toxin neutralizing assay

References

1. Klein U, la-Favera R. Germinal centres: role in B-cell physiology and malignancy. *Nat Rev Immunol.* 2008; 8:22–33. [PubMed: 18097447]
2. Weill JC, Weller S, Reynaud CA. Human marginal zone B cells. *Annu Rev Immunol.* 2009; 27:267–85. [PubMed: 19302041]
3. Schmidlin H, Diehl SA, Blom B. New insights into the regulation of human B-cell differentiation. *Trends Immunol.* 2009; 30:277–85. [PubMed: 19447676]
4. Ahlers JD, Belyakov IM. Memories that last forever: strategies for optimizing vaccine T-cell memory. *Blood.* 2010; 115:1678–89. [PubMed: 19903895]
5. Cockburn IA, Chen YC, Overstreet MG, et al. Prolonged antigen presentation is required for optimal CD8+ T cell responses against malaria liver stage parasites. *PLoS Pathog.* 2010; 6:e1000877. [PubMed: 20463809]
6. Guimond M, Veenstra RG, Grindler DJ, et al. Interleukin 7 signaling in dendritic cells regulates the homeostatic proliferation and niche size of CD4+ T cells. *Nat Immunol.* 2009; 10:149–57. [PubMed: 19136960]
7. Vezys V, Yates A, Casey KA, et al. Memory CD8 T-cell compartment grows in size with immunological experience. *Nature.* 2009; 457:196–9. [PubMed: 19005468]
8. Roach JC, Glusman G, Rowen L, et al. The evolution of vertebrate Toll-like receptors. *Proc Natl Acad Sci USA.* 2005; 102:9577–82. [PubMed: 15976025]
9. Vacchelli E, Eggermont A, Sautes-Fridman C, et al. Trial Watch: toll-like receptor agonists for cancer therapy. *Oncoimmunology.* 2013; 2:e25238. [PubMed: 24083080]
10. Eaton-Bassiri A, Dillon SB, Cunningham M, et al. Toll-like receptor 9 can be expressed at the cell surface of distinct populations of tonsils and human peripheral blood mononuclear cells. *Infect Immun.* 2004; 72:7202–11. [PubMed: 15557645]
11. Akira S, Takeda K. Functions of toll-like receptors: lessons from KO mice. *C R Biol.* 2004; 327:581–9. [PubMed: 15330257]
12. Tuomela J, Sandholm J, Karihtala P, et al. Low TLR9 expression defines an aggressive subtype of triple-negative breast cancer. *Breast Cancer Res Treat.* 2012; 135:481–93. [PubMed: 22847512]
13. Hagstrom J, Heikkila A, Siironen P, et al. TLR-4 expression and decrease in chronic inflammation: indicators of aggressive follicular thyroid carcinoma. *J Clin Pathol.* 2012; 65:333–8. [PubMed: 22267982]
14. Rakoff-Nahoum S, Medzhitov R. Toll-like receptors and cancer. *Nat Rev Cancer.* 2009; 9:57–63. [PubMed: 19052556]
15. Latz E, Schoenemeyer A, Visintin A, et al. TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nat Immunol.* 2004; 5:190–8. [PubMed: 14716310]
16. Krieg AM. Therapeutic potential of Toll-like receptor 9 activation. *Nat Rev Drug Discov.* 2006; 5:471–84. [PubMed: 16763660]
17. Hemmi H, Takeuchi O, Kawai T, et al. A Toll-like receptor recognizes bacterial DNA. *NAT.* 2000; 408:740–5.

18. Hornung V, Rothenfusser S, Britsch S, et al. Quantitative expression of toll-like receptor 1–10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J Immunol.* 2002; 168:4531–7. [PubMed: 11970999]
19. Suzuki Y, Wakita D, Chamoto K, et al. Liposome-encapsulated CpG oligodeoxynucleotides as a potent adjuvant for inducing type 1 innate immunity. *Cancer Res.* 2004; 64:8754–60. [PubMed: 15574787]
20. Kadowaki N, Ho S, Antonenko S, et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med.* 2001; 194:863–9. [PubMed: 11561001]
21. Bernasconi NL, Onai N, Lanzavecchia A. A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. *Blood.* 2003; 101:4500–4. [PubMed: 12560217]
22. Bourke E, Bosio D, Golay J, Polentarutti N, Mantovani A. The toll-like receptor repertoire of human B lymphocytes: inducible and selective expression of TLR9 and TLR10 in normal and transformed cells. *Blood.* 2003; 102:956–63. [PubMed: 12689944]
23. Browne EP. Regulation of B-cell responses by Toll-like receptors. *Immunology.* 2012; 136:370–9. [PubMed: 22444240]
24. Dement-Brown J, Newton CS, Ise T, Damdinsuren B, Nagata S, Tolnay M. Fc receptor-like 5 promotes B cell proliferation and drives the development of cells displaying switched isotypes. *J Leukoc Biol.* 2012; 91:59–67. [PubMed: 22028333]
25. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. *Int Rev Immunol.* 2011; 30:16–34. [PubMed: 21235323]
26. Banchereau J, Pascual V. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. *Immunity.* 2006; 25:383–92. [PubMed: 16979570]
27. Kaisho T. Pathogen sensors and chemokine receptors in dendritic cell subsets. *Vaccine.* 2012; 30:7652–7. [PubMed: 23142133]
28. Ewald SE, Lee BL, Lau L, et al. The ectodomain of Toll-like receptor 9 is cleaved to generate a functional receptor. *Nature.* 2008; 456:658–62. [PubMed: 18820679]
29. Park B, Brinkmann MM, Spooner E, Lee CC, Kim YM, Ploegh HL. Proteolytic cleavage in an endolysosomal compartment is required for activation of Toll-like receptor 9. *Nat Immunol.* 2008; 9:1407–14. [PubMed: 18931679]
30. Ewald SE, Engel A, Lee J, Wang M, Bogoy M, Barton GM. Nucleic acid recognition by Toll-like receptors is coupled to stepwise processing by cathepsins and asparagine endopeptidase. *J Exp Med.* 2011; 208:643–51. [PubMed: 21402738]
31. Sepulveda FE, Maschalidi S, Colisson R, et al. Critical role for asparagine endopeptidase in endocytic Toll-like receptor signaling in dendritic cells. *Immunity.* 2009; 31:737–48. [PubMed: 19879164]
32. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol.* 2004; 4:499–511. [PubMed: 15229469]
33. Lahoud MH, Ahmet F, Zhang JG, et al. DEC-205 is a cell surface receptor for CpG oligonucleotides. *Proc Natl Acad Sci USA.* 2012; 109:16270–5. [PubMed: 22988114]
34. Mutwiri GK, Nichani AK, Babiuk S, Babiuk LA. Strategies for enhancing the immunostimulatory effects of CpG oligodeoxynucleotides. *J Control Release.* 2004; 97:1–17. [PubMed: 15147800]
35. Verthelyi D, Ishii KJ, Gursel M, Takeshita F, Klinman DM. Human peripheral blood cells differentially recognize and respond to two distinct CpG motifs. *J Immunol.* 2001; 166:2372–7. [PubMed: 11160295]
36. Hartmann G, Battiany J, Poeck H, et al. Rational design of new CpG oligonucleotides that combine B cell activation with high IFN- α induction in plasmacytoid dendritic cells. *Eur J Immunol.* 2003; 33:1633–41. [PubMed: 12778481]
37. Krug A, Towarowski A, Britsch S, et al. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur J Immunol.* 2001; 31:3026–37. [PubMed: 11592079]

38. Guiducci C, Ott G, Chan JH, et al. Properties regulating the nature of the plasmacytoid dendritic cell response to Toll-like receptor 9 activation. *J Exp Med*. 2006; 203:1999–2008. [PubMed: 16864658]
39. Honda K, Ohba Y, Yanai H, et al. Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. *Nature*. 2005; 434:1035–40. [PubMed: 15815647]
40. Storni T, Ruedl C, Schwarz K, Schwendener RA, Renner WA, Bachmann MF. Nonmethylated CG motifs packaged into virus-like particles induce protective cytotoxic T cell responses in the absence of systemic side effects. *J Immunol*. 2004; 172:1777–85. [PubMed: 14734761]
41. Goldinger SM, Dummer R, Baumgaertner P, 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. [PubMed: 22806397]
42. Speiser DE, Schwarz K, Baumgaertner P, et al. Memory and effector CD8 T-cell responses after nanoparticle vaccination of melanoma patients. *J Immunother*. 2010; 33:848–58. [PubMed: 20842051]
43. Marshall JD, Fearon K, Abbate C, et al. Identification of a novel CpG DNA class and motif that optimally stimulate B cell and plasmacytoid dendritic cell functions. *J Leukoc Biol*. 2003; 73:781–92. [PubMed: 12773511]
44. Vollmer J, Weeratna R, Payette P, et al. Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities. *Eur J Immunol*. 2004; 34:251–62. [PubMed: 14971051]
45. Vollmer J, Krieg AM. Immunotherapeutic applications of CpG oligodeoxynucleotide TLR9 agonists. *Adv Drug Deliv Rev*. 2009; 61:195–204. [PubMed: 19211030]
46. Samulowitz U, Weber M, Weeratna R, et al. A novel class of immune-stimulatory CpG oligodeoxynucleotides unifies high potency in type I interferon induction with preferred structural properties. *Oligonucleotides*. 2010; 20:93–101. [PubMed: 20384481]
47. Weiner GJ, Liu HM, Wooldridge JE, Dahle CE, Krieg AM. Immunostimulatory oligodeoxynucleotides containing the CpG motif are effective as immune adjuvants in tumor antigen immunization. *Proc Natl Acad Sci USA*. 1997; 94:10833–7. [PubMed: 9380720]
48. Klinman DM, Currie D, Lee G, Grippe V, Merkel T. Systemic but not mucosal immunity induced by AVA prevents inhalational anthrax. *Microbes Infect*. 2007; 9:1478–83. [PubMed: 17913545]
49. Kamal S, Rashid AM, Bakar M, Ahad M. Anthrax: an update. *Asian Pac J Trop Biomed*. 2011; 1:496–501. [PubMed: 23569822]
50. Geier DA, Geier MR. Anthrax vaccination and joint related adverse reactions in light of biological warfare scenarios. *Clin Exp Rheumatol*. 2002; 20:217–20. [PubMed: 12051402]
51. Ready T. US soldiers refuse to fall in line with anthrax vaccination scheme. *Nat Med*. 2004; 10:112. [PubMed: 14760404]
52. Gu M, Hine PM, James JW, Giri L, Nabors GS. Increased potency of BioThrax anthrax vaccine with the addition of the C-class CpG oligonucleotide adjuvant CPG 10109. *Vaccine*. 2007; 25:526–34. [PubMed: 16973247]
53. Rynkiewicz D, Rathkopf M, Sim I, et al. Marked enhancement of the immune response to BioThrax(R) (Anthrax Vaccine Adsorbed) by the TLR9 agonist CPG 7909 in healthy volunteers. *Vaccine*. 2011; 29:6313–20. [PubMed: 21624418]
54. Hopkins RJ, Daczkowski NF, Kaptur PE, et al. Randomized, double-blind, placebo-controlled, safety and immunogenicity study of 4 formulations of Anthrax Vaccine Adsorbed plus CPG 7909 (AV7909) in healthy adult volunteers. *Vaccine*. 2013; 31:3051–8. [PubMed: 23701746]
55. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat*. 2004; 11:97–107. [PubMed: 14996343]
56. Bai JY, Yang YT, Zhu R, et al. CpG oligodeoxynucleotides discriminately enhance binding capacity of human naive B cells to Hepatitis B virus epitopes. *Can J Microbiol*. 2012; 58:752–9. [PubMed: 22625205]
57. Wang S, Han Q, Zhang G, et al. CpG oligodeoxynucleotide-adjuvanted fusion peptide derived from HBcAg epitope and HIV-Tat may elicit favorable immune response in PBMCs from patients with chronic HBV infection in the immunotolerant phase. *Int Immunopharmacol*. 2011; 11:406–11. [PubMed: 21182997]

58. McCluskie MJ, Davis HL. CpG DNA is a potent enhancer of systemic and mucosal immune responses against hepatitis B surface antigen with intranasal administration to mice. *J Immunol.* 1998; 161:4463–6. [PubMed: 9794366]
59. Davis HL, Suparto II, Weeratna RR, et al. CpG DNA overcomes hyporesponsiveness to hepatitis B vaccine in orangutans. *Vaccine.* 2000; 18:1920–4. [PubMed: 10699341]
60. Freed DC, Towne VM, Casimiro DR, Zhao Q, Fu TM. Evaluating functional antibodies in rhesus monkeys immunized with hepatitis B virus surface antigen vaccine with novel adjuvant formulations. *Vaccine.* 2011; 29:9385–90. [PubMed: 22001120]
61. Cooper CL, Davis HL, Morris ML, et al. CPG 7909, an immunostimulatory TLR9 agonist oligodeoxynucleotide, as adjuvant to Engerix-B HBV vaccine in healthy adults: a double-blind phase I/II study. *J Clin Immunol.* 2004; 24:693–701. [PubMed: 15622454]
62. Halperin SA, Van Nest G, Smith B, Abtahi S, Whiley H, Eiden JJ. A phase I study of the safety and immunogenicity of recombinant hepatitis B surface antigen co-administered with an immunostimulatory phosphorothioate oligonucleotide adjuvant. *Vaccine.* 2003; 21:2461–7. [PubMed: 12744879]
63. Halperin SA, Dobson S, McNeil S, et al. Comparison of the safety and immunogenicity of hepatitis B virus surface antigen co-administered with an immunostimulatory phosphorothioate oligonucleotide and a licensed hepatitis B vaccine in healthy young adults. *Vaccine.* 2006; 24:20–6. [PubMed: 16198027]
64. Halperin SA, Ward B, Cooper C, et al. Comparison of safety and immunogenicity of two doses of investigational hepatitis B virus surface antigen co-administered with an immunostimulatory phosphorothioate oligodeoxyribonucleotide and three doses of a licensed hepatitis B vaccine in healthy adults 18–55 years of age. *Vaccine.* 2012; 30:2556–63. [PubMed: 22326642]
65. Sablan BP, Kim DJ, Barzaga NG, et al. Demonstration of safety and enhanced seroprotection against hepatitis B with investigational HBsAg-1018 ISS vaccine compared to a licensed hepatitis B vaccine. *Vaccine.* 2012; 30:2689–96. [PubMed: 22342916]
66. Gupta S, Gunter JT, Novak RJ, Regens JL. Patterns of *Plasmodium vivax* and *Plasmodium falciparum* malaria underscore importance of data collection from private health care facilities in India. *Malar J.* 2009; 8:227. [PubMed: 19822005]
67. Ellis RD, Sagara I, Doumbo O, Wu Y. Blood stage vaccines for *Plasmodium falciparum*: current status and the way forward. *Hum Vaccin.* 2010; 6:627–34. [PubMed: 20519960]
68. Langhorne J, Ndungu FM, Sponaas AM, Marsh K. Immunity to malaria: more questions than answers. *Nat Immunol.* 2008; 9:725–32. [PubMed: 18563083]
69. Lanzavecchia A, Bernasconi N, Traggiai E, Ruprecht CR, Corti D, Sallusto F. Understanding and making use of human memory B cells. *Immunol Rev.* 2006; 211:303–9. [PubMed: 16824137]
70. Sanz I, Wei C, Lee FE, Anolik J. Phenotypic and functional heterogeneity of human memory B cells. *Semin Immunol.* 2008; 20:67–82. [PubMed: 18258454]
71. Kennedy MC, Wang J, Zhang Y, et al. In vitro studies with recombinant *Plasmodium falciparum* apical membrane antigen 1 (AMA1): production and activity of an AMA1 vaccine and generation of a multiallelic response. *Infect Immun.* 2002; 70:6948–60. [PubMed: 12438374]
72. Dutta S, Sullivan JS, Grady KK, et al. High antibody titer against apical membrane antigen-1 is required to protect against malaria in the Aotus model. *PLoS One.* 2009; 4:e8138. [PubMed: 19997632]
73. Dicko A, Diemert DJ, Sagara I, et al. Impact of a *Plasmodium falciparum* AMA1 vaccine on antibody responses in adult Malians. *PLoS One.* 2007; 2:e1045. [PubMed: 17940609]
74. Sagara I, Ellis RD, Dicko A, et al. A randomized and controlled Phase 1 study of the safety and immunogenicity of the AMA1-C1/Alhydrogel + CPG 7909 vaccine for *Plasmodium falciparum* malaria in semi-immune Malian adults. *Vaccine.* 2009; 27:7292–8. [PubMed: 19874925]
75. Crompton PD, Mircetic M, Weiss G, et al. The TLR9 ligand CpG promotes the acquisition of *Plasmodium falciparum*-specific memory B cells in malaria-naïve individuals. *J Immunol.* 2009; 182:3318–26. [PubMed: 19234231]
76. Mullen GE, Ellis RD, Miura K, et al. Phase 1 trial of AMA1-C1/Alhydrogel plus CPG 7909: an asexual blood-stage vaccine for *Plasmodium falciparum* malaria. *PLoS One.* 2008; 3:e2940. [PubMed: 18698359]

77. Ellis RD, Martin LB, Shaffer D, et al. Phase 1 trial of the Plasmodium falciparum blood stage vaccine MSP1(42)-C1/Alhydrogel with and without CPG 7909 in malaria naive adults. *PLoS One*. 2010; 5:e8787. [PubMed: 20107498]
78. Duncan CJ, Sheehy SH, Ewer KJ, et al. Impact on malaria parasite multiplication rates in infected volunteers of the protein-in-adjuvant vaccine AMA1-C1/Alhydrogel + CPG 7909. *PLoS One*. 2011; 6:e22271. [PubMed: 21799809]
79. Moldoveanu Z, Love-Homan L, Huang WQ, Krieg AM. CpG DNA, a novel immune enhancer for systemic and mucosal immunization with influenza virus. *Vaccine*. 1998; 16:1216–24. [PubMed: 9682382]
80. Rhee JW, Kim D, Park BK, et al. Immunization with a hemagglutinin-derived synthetic peptide formulated with a CpG-DNA-liposome complex induced protection against lethal influenza virus infection in mice. *PLoS One*. 2012; 7:e48750. [PubMed: 23144954]
81. Fang Y, Rowe T, Leon AJ, et al. Molecular characterization of in vivo adjuvant activity in ferrets vaccinated against influenza virus. *J Virol*. 2010; 84:8369–88. [PubMed: 20534862]
82. Cooper CL, Davis HL, Morris ML, et al. Safety and immunogenicity of CPG 7909 injection as an adjuvant to Fluorix influenza vaccine. *Vaccine*. 2004; 22:3136–43. [PubMed: 15297066]
83. Lopez-Palomo C, Martin-Zamorano M, Benitez E, et al. Pneumonia in HIV-infected patients in the HAART era: incidence, risk, and impact of the pneumococcal vaccination. *J Med Virol*. 2004; 72:517–24. [PubMed: 14981752]
84. Hersperger AR, Pereyra F, Nason M, et al. Perforin expression directly ex vivo by HIV-specific CD8 T-cells is a correlate of HIV elite control. *PLoS Pathog*. 2010; 6:e1000917. [PubMed: 20523897]
85. Agrawal S, Agrawal A, Doughty B, et al. Cutting edge: different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *J Immunol*. 2003; 171:4984–9. [PubMed: 14607893]
86. Scheller C, Ullrich A, McPherson K, et al. CpG oligodeoxynucleotides activate HIV replication in latently infected human T cells. *J Biol Chem*. 2004; 279:21897–902. [PubMed: 15016800]
87. Equils O, Schito ML, Karahashi H, et al. Toll-like receptor 2 (TLR2) and TLR9 signaling results in HIV-long terminal repeat trans-activation and HIV replication in HIV-1 transgenic mouse spleen cells: implications of simultaneous activation of TLRs on HIV replication. *J Immunol*. 2003; 170:5159–64. [PubMed: 12734363]
88. Sulkowski MS, Chaisson RE, Karp CL, Moore RD, Margolick JB, Quinn TC. The effect of acute infectious illnesses on plasma human immunodeficiency virus (HIV) type 1 load and the expression of serologic markers of immune activation among HIV-infected adults. *J Infect Dis*. 1998; 178:1642–8. [PubMed: 9815216]
89. Rotchford K, Strum AW, Wilkinson D. Effect of coinfection with STDs and of STD treatment on HIV shedding in genital-tract secretions: systematic review and data synthesis. *Sex Transm Dis*. 2000; 27:243–8. [PubMed: 10821594]
90. Cooper CL, Davis HL, Angel JB, et al. CPG 7909 adjuvant improves hepatitis B virus vaccine seroprotection in antiretroviral-treated HIV-infected adults. *AIDS*. 2005; 19:1473–9. [PubMed: 16135900]
91. Angel JB, Cooper CL, Clinch J, et al. CpG increases vaccine antigen-specific cell-mediated immunity when administered with hepatitis B vaccine in HIV infection. *J Immune Based Ther Vaccines*. 2008; 6:4. [PubMed: 18700037]
92. Cooper CL, Angel JB, Seguin I, Davis HL, Cameron DW. CPG 7909 adjuvant plus hepatitis B virus vaccination in HIV-infected adults achieves long-term seroprotection for up to 5 years. *Clin Infect Dis*. 2008; 46:1310–4. [PubMed: 18444872]
93. Sogaard OS, Lohse N, Harboe ZB, et al. Improving the immunogenicity of pneumococcal conjugate vaccine in HIV-infected adults with a toll-like receptor 9 agonist adjuvant: a randomized, controlled trial. *Clin Infect Dis*. 2010; 51:42–50. [PubMed: 20504165]
94. Offersen R, Melchjorsen J, Paludan SR, Ostergaard L, Tolstrup M, Sogaard OS. TLR9-adjuvanted pneumococcal conjugate vaccine induces antibody-independent memory responses in HIV-infected adults. *Hum Vaccin Immunother*. 2012; 8:1042–7. [PubMed: 22854665]

95. Winckelmann AA, Munk-Petersen LV, Rasmussen TA, et al. Administration of a Toll-like receptor 9 agonist decreases the proviral reservoir in virologically suppressed HIV-infected patients. *PLoS One*. 2013; 8:e62074. [PubMed: 23637967]
96. Krieg AM. Antitumor applications of stimulating toll-like receptor 9 with CpG oligodeoxynucleotides. *Curr Oncol Rep*. 2004; 6:88–95. [PubMed: 14751085]
97. Carpentier AF, Chen L, Maltonti F, Delattre JY. Oligodeoxynucleotides containing CpG motifs can induce rejection of a neuroblastoma in mice. *Cancer Res*. 1999; 59:5429–32. [PubMed: 10554011]
98. Carpentier AF, Xie J, Mokhtari K, Delattre JY. Successful treatment of intracranial gliomas in rat by oligodeoxynucleotides containing CpG motifs. *Clin Cancer Res*. 2000; 6:2469–73. [PubMed: 10873101]
99. 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. [PubMed: 22231700]
100. Carpentier A, Laigle-Donadey F, Zohar S, et al. Phase 1 trial of a CpG oligodeoxynucleotide for patients with recurrent glioblastoma. *Neuro Oncol*. 2006; 8:60–6. [PubMed: 16443949]
101. Carpentier A, Metellus P, Ursu R, et al. Intracerebral administration of CpG oligonucleotide for patients with recurrent glioblastoma: a phase II study. *Neuro Oncol*. 2010; 12:401–8. [PubMed: 20308317]
102. Lienard D, Rimoldi D, Marchand M, et al. Ex vivo detectable activation of Melan-A-specific T cells correlating with inflammatory skin reactions in melanoma patients vaccinated with peptides in IFA. *Cancer Immun*. 2004; 4:4. [PubMed: 15149168]
103. Cormier JN, Salgaller ML, Prevette T, et al. Enhancement of cellular immunity in melanoma patients immunized with a peptide from MART-1/Melan A. *Cancer J Sci Am*. 1997; 3:37–44. [PubMed: 9072306]
104. Ramos TC, Vinageras EN, Ferrer MC, et al. Treatment of NSCLC patients with an EGF-based cancer vaccine: report of a Phase I trial. *Cancer Biol Ther*. 2006; 5:145–9. [PubMed: 16357522]
105. Gonzalez G, Crombet T, Catala M, et al. A novel cancer vaccine composed of human-recombinant epidermal growth factor linked to a carrier protein: report of a pilot clinical trial. *Ann Oncol*. 1998; 9:431–5. [PubMed: 9636835]
106. Perales MA, Yuan J, Powel S, et al. Phase I/II study of GM-CSF DNA as an adjuvant for a multipptide cancer vaccine in patients with advanced melanoma. *Mol Ther*. 2008; 16:2022–9. [PubMed: 18797450]
107. Krug LM, Dao T, Brown AB, et al. WT1 peptide vaccinations induce CD4 and CD8 T cell immune responses in patients with mesothelioma and non-small cell lung cancer. *Cancer Immunol Immunother*. 2010; 59:1467–79. [PubMed: 20532500]
108. Jager E, Nagata Y, Gnjatich S, et al. Monitoring CD8 T cell responses to NY-ESO-1: correlation of humoral and cellular immune responses. *Proc Natl Acad Sci USA*. 2000; 97:4760–5. [PubMed: 10781081]
109. Baumgaertner P, Rufer N, Devereux E, et al. Ex vivo detectable human CD8 T-cell responses to cancer-testis antigens. *Cancer Res*. 2006; 66:1912–6. [PubMed: 16488988]
110. Barve M, Bender J, Senzer N, et al. Induction of immune responses and clinical efficacy in a phase II trial of IDM-2101, a 10-epitope cytotoxic T-lymphocyte vaccine, in metastatic non-small-cell lung cancer. *J Clin Oncol*. 2008; 26:4418–25. [PubMed: 18802154]
111. Vansteenkiste J, Zielinski M, Linder A, et al. Adjuvant MAGE-A3 immunotherapy in resected non-small-cell lung cancer: phase II randomized study results. *J Clin Oncol*. 2013; 31:2396–403. [PubMed: 23715567]
112. Bode C, Zhao G, Steinhagen F, Kinjo T, Klinman DM. CpG DNA as a vaccine adjuvant. *Expert Rev Vaccines*. 2011; 10:499–511. [PubMed: 21506647]
113. Shirota H, Klinman DM. Recent progress concerning CpG DNA and its use as a vaccine adjuvant. *Expert Rev Vaccines*. 2014; 13:299–312. [PubMed: 24308579]
114. Ursu R, Carpentier AF. Immunotherapeutic approach with oligodeoxynucleotides containing CpG motifs (CpG-ODN) in malignant glioma. *Adv Exp Med Biol*. 2012; 746:95–108. [PubMed: 22639162]

115. Baumgaertner P, Jandus C, Rivals JP, et al. Vaccination-induced functional competence of circulating human tumor-specific CD8 T-cells. *Int J Cancer*. 2012; 130:2607–17. [PubMed: 21796616]
116. Tarhini AA, Leng S, Moschos SJ, et al. Safety and immunogenicity of vaccination with MART-1 (26-35, 27L), gp100 (209-217, 210M), and tyrosinase (368-376, 370D) in adjuvant with PF-3512676 and GM-CSF in metastatic melanoma. *J Immunother*. 2012; 35:359–66. [PubMed: 22495394]
117. Del VM, Di GL, Ascierto PA, et al. Efficacy and safety of ipilimumab 3 mg/kg in patients with pretreated, metastatic, mucosal melanoma. *Eur J Cancer*. 2014; 50:121–7. [PubMed: 24100024]
118. Ohno S, Okuyama R, Aruga A, Sugiyama H, Yamamoto M. Phase I trial of Wilms' Tumor 1 (WT1) peptide vaccine with GM-CSF or CpG in patients with solid malignancy. *Anticancer Res*. 2012; 32:2263–9. [PubMed: 22641661]
119. Heikenwalder M, Polymenidou M, Junt T, et al. Lymphoid follicle destruction and immunosuppression after repeated CpG oligodeoxynucleotide administration. *Nat Med*. 2004; 10:187–92. [PubMed: 14745443]
120. Sparwasser T, Hultner L, Koch ES, Luz A, Lipford GB, Wagner H. Immunostimulatory CpG-oligodeoxynucleotides cause extramedullary murine hemopoiesis. *J Immunol*. 1999; 162:2368–74. [PubMed: 9973517]
121. Krieg AM, Efler SM, Wittpoth M, Al Adhami MJ, Davis HL. Induction of systemic TH1-like innate immunity in normal volunteers following subcutaneous but not intravenous administration of CPG 7909, a synthetic B-class CpG oligodeoxynucleotide TLR9 agonist. *J Immunother*. 2004; 27:460–71. [PubMed: 15534490]
122. Leonard JP, Link BK, Emmanouilides C, et al. Phase I trial of toll-like receptor 9 agonist PF-3512676 with and following rituximab in patients with recurrent indolent and aggressive non Hodgkin's lymphoma. *Clin Cancer Res*. 2007; 13:6168–74. [PubMed: 17947483]
123. Manegold C, Gravenor D, Woytowitz D, et al. Randomized phase II trial of a toll-like receptor 9 agonist oligodeoxynucleotide, PF-3512676, in combination with first-line taxane plus platinum chemotherapy for advanced-stage non-small-cell lung cancer. *J Clin Oncol*. 2008; 26:3979–86. [PubMed: 18711188]
124. Witzig TE, Wiseman GA, Maurer MJ, et al. A phase I trial of immunostimulatory CpG 7909 oligodeoxynucleotide and (90) yttrium ibritumomab tiuxetan radioimmunotherapy for relapsed B-cell non-Hodgkin lymphoma. *Am J Hematol*. 2013; 88:589–93. [PubMed: 23619698]
125. Krieg AM, Vollmer J. Toll-like receptors 7, 8, and 9: linking innate immunity to autoimmunity. *Immunol Rev*. 2007; 220:251–69. [PubMed: 17979852]
126. Ellis RD, Mullen GE, Pierce M, et al. A Phase 1 study of the blood-stage malaria vaccine candidate AMA1-C1/Alhydrogel with CPG 7909, using two different formulations and dosing intervals. *Vaccine*. 2009; 27:4104–9. [PubMed: 19410624]
127. Kim YH, Girardi M, Duvic M, et al. Phase I trial of a Toll-like receptor 9 agonist, PF-3512676 (CPG 7909), in patients with treatment-refractory, cutaneous T-cell lymphoma. *J Am Acad Dermatol*. 2010; 63:975–83. [PubMed: 20888065]
128. Karbach J, Neumann A, Atmaca A, et al. Efficient in vivo priming by vaccination with recombinant NY-ESO-1 protein and CpG in antigen naive prostate cancer patients. *Clin Cancer Res*. 2011; 17:861–70. [PubMed: 21163871]
129. Speiser DE, Lienard D, Rufer N, et al. Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. *J Clin Invest*. 2005; 115:739–46. [PubMed: 15696196]
130. Schlom J. Recombinant cancer vaccines and new vaccine targets. *Expert Rev Vaccines*. 2013; 12:1121–4. [PubMed: 24098990]
131. Fujiki F, Oka Y, Kawakatsu M, et al. A clear correlation between WT1-specific Th response and clinical response in WT1 CTL epitope vaccination. *Anticancer Res*. 2010; 30:2247–54. [PubMed: 20651376]
132. Klebanoff CA, Gattinoni L, Restifo NP. CD8+ T-cell memory in tumor immunology and immunotherapy. *Immunol Rev*. 2006; 211:214–24. [PubMed: 16824130]

133. Hara I, Takechi Y, Houghton AN. Implicating a role for immune recognition of self in tumor rejection: passive immunization against the brown locus protein. *J Exp Med.* 1995; 182:1609–14. [PubMed: 7595233]
134. Madan RA, Bilusic M, Heery C, Schlom J, Gulley JL. Clinical evaluation of TRI-COM vector therapeutic cancer vaccines. *Semin Oncol.* 2012; 39:296–304. [PubMed: 22595052]
135. Miura K, Zhou H, Moretz SE, et al. Comparison of biological activity of human anti-apical membrane antigen-1 antibodies induced by natural infection and vaccination. *J Immunol.* 2008; 181:8776–83. [PubMed: 19050299]
136. Traore B, Kone Y, Doumbo S, et al. The TLR9 agonist CpG fails to enhance the acquisition of *Plasmodium falciparum*-specific memory B cells in semi-immune adults in Mali. *Vaccine.* 2009; 27:7299–303. [PubMed: 19712767]
137. Ellis RD, Wu Y, Martin LB, et al. Phase 1 study in malaria naive adults of BSAM2/ Alhydrogel(R) + CPG 7909, a blood stage vaccine against *P. falciparum* malaria. *PLoS One.* 2012; 7:e46094. [PubMed: 23056238]
138. La RC, Longmate J, Lacey SF, et al. Clinical evaluation of safety and immunogenicity of PADRE-cytomegalovirus (CMV) and tetanus-CMV fusion peptide vaccines with or without PF03512676 adjuvant. *J Infect Dis.* 2012; 205:1294–304. [PubMed: 22402037]
139. Halperin SA, McNeil S, Langley JM, et al. Safety and immunogenicity of different two-dose regimens of an investigational hepatitis B vaccine (hepatitis B surface antigen co-administered with an immunostimulatory phosphorothioate oligodeoxyribonucleotide) in healthy young adults. *Vaccine.* 2012; 30:5445–8. [PubMed: 22704926]
140. Fourcade J, Kudela P, Andrade Filho PA, et al. Immunization with analog peptide in combination with CpG and montanide expands tumor antigen-specific CD8+ T cells in melanoma patients. *J Immunother.* 2008; 31:781–91. [PubMed: 18779741]
141. Valmori D, Souleimanian NE, Tosello V, et al. Vaccination with NY-ESO-1 protein and CpG in Montanide induces integrated antibody/Th1 responses and CD8 T cells through cross-priming. *Proc Natl Acad Sci USA.* 2007; 104:8947–52. [PubMed: 17517626]
142. Karbach J, Gnjjatic S, Bender A, et al. Tumor-reactive CD8+ T-cell responses after vaccination with NY-ESO-1 peptide, CpG 7909 and Montanide ISA-51: association with survival. *Int J Cancer.* 2010; 126:909–18. [PubMed: 19728336]
143. Iwahashi M, Katsuda M, Nakamori M, et al. Vaccination with peptides derived from cancer-testis antigens in combination with CpG-7909 elicits strong specific CD8+ T cell response in patients with metastatic esophageal squamous cell carcinoma. *Cancer Sci.* 2010; 101:2510–7. [PubMed: 20874827]

Table 1

CpG ODN as adjuvants in infectious disease trials.

Trial no.	Infection	Treatment	Route	Phase	Date	Results	Reference
NCT00344539	Malaria	AMAI-C1/Alhydrogel® (20, 80 µg) ± CpG 7909 (564 µg) <i>n</i> = 75 in 3 groups days: 0, 28, 56	IM	I	04/05–03/07	AMA-1 + CpG vaccination supported an 11–14 fold increase in anti-AMA1 Ab titers (<i>p</i> < 0.02), PF GIA was 3–4 fold higher (<i>p</i> < 0.0001) than AMA-1 alone, but similar growth inhibitory activity against PF in standardized AMA1-IgG titer.	[76,135]
NCT00414336	Malaria	AMAI-C1/Alhydrogel® (80 µg) ± CpG 7909 (564 µg) <i>n</i> = 24 in 2 groups days: 0, 28	IM	I	10/07–11/07	AMA-1 + CpG vaccination of malaria exposed adults increased AMA1-specific Abs by 2–3 fold vs. AMA-1 alone; no significant differences in Ag specific memory B cells or GIA between groups.	[74,136]
NCT00320658	Malaria	AMAI-C1/Alhydrogel® (20, 80 µg) + MSP1 ₄₂ -C1/Alhydrogel® (80 µg) ± CpG 7909 (564 µg) <i>n</i> = 24 in 2 groups days: 0, 28, 56	IM	I	07/06–07/07	CpG 7909 enhanced significantly the appearance or AMA1-C1-specific memory B cells (2–10 fold at days 35, 56, 59; <i>p</i> < 0.01) and MSP1 ₄₂ -specific memory B cells (7–10 fold at day 3 and 84 after 3rd vaccination) in PBMC.	[75,126]
NCT00984763	Malaria	AMAI-C1/Alhydrogel® (80 µg) ± CpG 7909 (564 µg) <i>n</i> = 10 in 2 groups days: 0, 28, 56	IM	I/IIa	07/09–09/10	Inclusion of CpG significantly increased GIA titers (63% versus 13% <i>p</i> < 0.01) and correlated with lower PMR.	[78]
NCT00889616	Malaria	BSAM2/Alhydrogel® (40, 160 µg) + CPG 7909 (564 µg) <i>n</i> = 30 in 2 groups days: 0, 56, 180	IM	I	06/09–03/10	Both doses of BSAM2/Alhydrogel® + CpG 7909 were immunogenic; increase of anti-AMA1 (86 and 113 µg/mL) and anti-MSP ₄₂ titers (57 and 76 µg/mL) Ab at day 194 with boosting effect after the second vaccination; GIA increased 7–41%.	[137]
NA	Influenza	Fluarix® (0.5, 0.05 mL) ± CpG 7909 (1 mg) <i>n</i> = 60 in 4 groups Single dose	IM	Ib	08/99–10/99	CpG 7909 did not increase anti-H1 Ab titers vs. vaccine alone (<i>p</i> > 0.05). Upon Ag restimulation, PBMC at 4 week showed significantly higher IFN-γ production for A/Beijing/262/95 (<i>p</i> = 0.048) and B/Harbin/7/94 (<i>p</i> = 0.0057) in 1/10th dose Fluarix® + CpG 7909 compared to 1/10th dose Fluarix® alone.	[82]
NCT00562939	Pneumococcus in HIV-infected adults	PCV7 (Prevnar®), PPV-23 (PneumoNovum®) (1 mL) ± CPG 7909 (1 mg) <i>n</i> = 97 in 2 groups 0, 3, 9 months	IM	Ib/IIa	01/08–01/09	CpG group showed a mean reduction in proviral DNA of 12.6% vs. a 6.7% increase in the non-CpG group (<i>p</i> = 0.02). No significant changes in HIV specific Ab or T cell immunity. PBMC from the CpG group produced significantly more IL-1β (<i>p</i> = 0.0046), IL-6 (<i>p</i> = 0.0051), IFN-γ (<i>p</i> = 0.0047), MIP-β (<i>p</i> = 0.0086), IL-2R (<i>p</i> = 0.0062) levels. PBMC of CpG vaccinated patients stimulated with LPS showed elevated production of L-1β (<i>p</i> = 0.038) and IFN-γ (<i>p</i> = 0.019) vs. vaccine alone.	[93–95]
NCT00722839	Cytomegalovirus (CMV)	PADRE-CMV (1.5, 2.5, 10 mg), Tetanus-CMV fusion peptide, ±CpG 7909 (1 mg) <i>n</i> = 63 in 4 groups days: 0, 21, 42, 63	SQ	Ib	05/10–10/10	CMV alone induced no response, CD8 T cell responses induced in 30% of CpG plus PADRE-CMV and 70% of Tetanus-CMV plus CpG groups (<i>p</i> = 0.002), pp65 specific CD8 T cells increased 5–10 fold in tetramere binding assay compared to vaccine alone (<i>p</i> = 0.004) and 4–5 fold vs. prevaccination levels (<i>p</i> = 0.008).	[138]

Trial no.	Infection	Treatment	Route	Phase	Date	Results	Reference
NA	HBV	HBsAg (20 µg) + 1018 ISS (300, 650, 1000, 3000 µg) <i>n</i> = 48 in 6 groups 0, 8 wk	IM	I	NA	Seroprotective titers (>10 mIU/L) were reached by 0, 25, 75 and 87% of patients after 1st vaccination vs. 62, 100, 100% after 2nd vaccination with increasing CpG ODN doses. GMA anti-HBsAg were 1.22, 5.78, 24.75, 206.5 after 1st and 65.37, 877.6, 1545, 3045 mIU/mL after 2nd vaccination. Injection site reactions were more frequent in CpG group (<i>p</i> 0.05) but all doses were well tolerate.	[62]
NA	HBV	rHBsAg (20 µg) + 1018 ISS (3 mg), Engerix-B® <i>n</i> = 99 in 2 groups 0, 8, 24 weeks	IM	II	NA	CpG increased the percentage of participants achieving seroprotection after 1st, 2nd and 3rd vaccination compared to Engerix-B® alone (79% vs. 12%; 100% vs. 18%; 100% vs. 64%; <i>p</i> < 0.001). GMC of anti-HBsAg Ab were higher in CpG group at week 1, 4, 8 and 16 after the 1st and 2nd dose (<i>p</i> < 0.001).	[63]
NA	HBV	rHBsAg (20 µg) + 1018 ISS (3 mg), Engerix-B® (1 mL) <i>n</i> = 2415 in 2 groups 0, 4 or 0, 4, 24 weeks	IM	III	12/06–07/07	Higher anti-HBV titers and improved seroprotection was achieved in the CpG group at 4, 8, 24 and 28 weeks post vaccination (23% vs. 3%; 88% vs. 26%; 98% vs. 32%; 97% vs. 81%). GMC were >20-fold higher after the 2nd vaccination and at similar levels as Engerix-B® group after the 3rd vaccination.	[64]
NA	HBV	rHBsAg (20 µg) + 1018 ISS (3 mg), <i>n</i> = 41 in 2 groups 0, 4 or 0, 8 week	IM	III	NA	Seroprotective anti-HBsAg Ab titers (10 mIU/mL) were higher in the CpG adjuvanted group (94% vs. 69%) at week 4. GMC levels were 15-fold higher at 4 week (243 vs. 16 mIU/mL; <i>p</i> < 0.001). Difference between groups diminished with boosting but persisted through week 32 (863 vs. 439 mIU/mL; <i>p</i> = 0.038).	[139]
NA	HBV in adults 40–70 years	rHBsAg (20 µg) + 1018 ISS (3 mg), Engerix-B® (1 mL) <i>n</i> = 409 in 2 groups 0, 4, 8, 24 weeks	IM	III	NA	Seroprotection achieved in more CpG recipients at 4 and 28 week (96% vs. 24%; 100% vs. 73%; <i>p</i> < 0.0001). Titers of anti-HBsAg >100 mIU/mL were induced in 97% vs. 58% at week 28 and in 90% vs. 43% at week 50 compared to Engerix-B® (<i>p</i> < 0.0001) as well as significantly higher GMC were seen at these time points (<i>p</i> < 0.0001).	[65]
NA	HBV	Engerix-B® (20 µg HBsAg) ± CpG 7909 (0.125, 0.5, 1 mg) <i>n</i> = 56 in 4 groups 0, 4, 24 weeks	IM	I/II	04/99–06/01	Inclusion of CpG accelerated the development of protective anti-HBsAg Ab titers (<i>p</i> < 0.001) vs. vaccine alone. The 0.5 mg dose of CpG 7909 was most effective (2-fold higher than 0.125 or 1.0 mg, <i>p</i> < 0.05).	[61]
NA	HBV in HIV-infected adults	Engerix-B® (40 µg HBsAg) + CpG 7909 (0.5, 1 mg) <i>n</i> = 58 in 4 groups (<i>n</i> = 38 in 2 groups) 0, 4, 8 week	IM	Ib/Ila	01/01–08/02	Inclusion of CpG significantly increased anti-HBsAg Ab titers from 6 to 48 weeks. T helper cells showed higher proliferative response to HBsAg ex vivo at week 8 (<i>p</i> = 0.042) and 48 (<i>p</i> = 0.024). No changes in CD4 or CD8 T cell subsets through 5 years of follow up. The CpG group retained seroprotective Anti-ABsAg titers at 60 months (80% vs. 40%, <i>p</i> = 0.004).	[90–92]
NA	Anthrax	Bio-Thrax® (0.5 mL), ±CpG 7909 (1 mg) <i>n</i> = 69 in 3 groups days: 0, 14, 28	IM	I	NA	BioThrax® + CpG 7909 induced 6 fold higher IgG anti-PA Ab titers (1465 µg/mL vs. 252 µg/mL, <i>p</i> < 0.001) that peaked earlier (day 21 vs. 40, <i>p</i> < 0.001) vs. vaccine alone. Toxin neutralizing capacity was 8.8 fold higher (<i>p</i> < 0.001) and also developed earlier (day 22 vs. 46, <i>p</i> < 0.001).	[53]

Trial no.	Infection	Treatment	Route	Phase	Date	Results	Reference
NCT01263691	Anthrax	Bio-Thrax® (0.5 mL), AV7909: AVA (0.25–0.5 mL) + CpG 7909 (0.25–0.5 mg) <i>n</i> = 105 in 4 groups days: 0, 14	IM	I	NA	Inclusion of any dose of AV 7909 yielded 2-fold higher GMT in TNA NF50 (50% neutralization factor), CpG accelerated reaching peak titer (day 28 vs. 35).	[54]

Table 2

CpG ODN as adjuvant in tumor vaccine trials.

Trial no.	Type of cancer	Treatment	Route	Phase	Date	Results	Reference
NA	Malignant melanoma	2/Melan-A (100 µg), Montanide® ISA-51 (300 µg), +CpG 7909 (500 µg) <i>n</i> = 16 in 2 group 4× monthly	SQ	I	NA	CpG group: more rapid (after 2 vs. 4 vaccinations) and 8-fold higher Melan-A-specific CD8 T cell response compared to the non-CpG group ($1.15 \pm 0.93\%$ vs. $0.13 \pm 0.11\%$, $p < 0.01$). Response rate was 100% after 4 vaccinations vs. 50% in controls. Clinical response: one patient (12%) remained disease free, one patient SD, 6 patients (75%) PD.	[129]
NCT000085189	Malignant melanoma stage III–IV	NY-ESO-1 (600 µg), Montanide® ISA-51 (3 mL), ±CpG 7909 (2 mg) <i>n</i> = 8 in 3 groups 6× biweekly	SQ	II	05/04–09/07	CpG group: 100% of patients (<i>n</i> = 3) developed NY-ESO-1 specific CD8 T cells which increased 3–10 fold after 6 vaccinations compared to pre-vaccination status. CpG alone (without NY-ESO-1) had no effect. Clinical outcome: 1 (12%) patient had SD for 6 months then progressed as did all other patients.	[140]
NCT00306514, 00306566, 00306553	Malignant melanoma stage II–IV	CYT004-MelQbG10 (1 mg) <i>n</i> = 22 in 4 groups, 6 or 11 vaccinations, (wkly or daily 5x)	SQ/ID	I/II	2006	Significant increase in Ag-specific T cells in 14/22 patients (63%) compared to pre-vaccination status (no differences between groups I–IV). T cells produced TNF- α , IFN- γ and IL-2 and up-regulated expression of surface antigens LAMP-1 and CD107a. Significantly more memory T cells vs. seen patients vaccinated with Melan-A peptide plus IFA ($p < 0.05$) from study [102]. Clinical response: 7 (31%) patients were free of disease, 1 patient (4%) PR, 11 patients (50%) PD, 1 patient (4%) SD, 2 (9%) patients died.	[42]
NCT00651703	Malignant melanoma stage III–IV	CYT004-MelQbG10, Montanide® ISA-51, Imiquimod 5%, <i>n</i> = 21 in 4 groups 3x wkly, 3x monthly	SQ/ID/IN	I/IIa	04/08–07/10	76% of patients responded to MelQbG10 vaccination by a >2 fold increase in Ag-specific T cells. Clinical response: 5 patients (23.8%) had SD, 9 patients (42%) PD, 5 (23%) patients without evidence of disease, 2 (9%) patients died.	[41]
NCT00112229	Malignant melanoma stage III–IV	Melan-A/Mart-1, Tyrosinase (YMD) Montanide® ISA-51 ± CpG 7909 <i>n</i> = 24 in 2 groups monthly	SQ	I	04/03–06/12	CpG group: Melan-A/MART-1 specific T cell frequencies were 10-fold higher ($p < 0.01$) and produced higher levels of IFN- γ , TNF- α and IL-2 ($p < 0.01$) compared to non-CpG group.	[115]

Trial no.	Type of cancer	Treatment	Route	Phase	Date	Results	Reference
NA	Malignant melanoma stage III–IV	MART-1, gp100, tyrosinase peptides, Montanide® ISA-51, + CpG 7909 (600 µg), GM-CSF (80 µg) n = 22 one group days: 1, 15 (13 cycles)	SQ	I	01/09–12/10	2 (9.5%) patients had PR, 8 (38%) patients with SD (2–7 months, 50% of them with positive Elispot), 20 (95%) patients had PD in follow up; median PFS 1.87 months, median OS 13.4 months.	[116]
NA	Multiple types (melanoma, breast cancer, ovarian cancer, sarcoma) stages I–IV	rNY-ESO-1 (100, 400 µg), Montanide® ISA-51, + CpG 7909 (2.5 mg), n = 18 in 2 groups 4 × 3 week interval	SQ	NA	NA	94% of patients developed CD4 T cell responses and 50% CD8 T cell responses. IgG Ab titer of 24 ± 10 in those vaccinated with 100 µg NY-ESO-1; 42 ± 29 in the 400 µg NY-ESO-1 group (preferentially IgG1 and IgG3).	[141]
NCT00199836	Multiple types (melanoma, NSCLC, breast cancer, ovarian cancer, sarcoma) stage III–IV	NY-ESO-1b (100 µg), Montanide® ISA-51 (0.5 mL), +CpG 7909 (1 mg) n = 14 one group 4 × 3 week interval	SQ	I	09/03–12/05	Induction of Ag-specific CD8 T cells against NY-ESO-1 in 9 of 14 patients (63%). Clinical response: 2 patients (14%) showed no evidence of disease, 9 patients (64%) had PD, 3 patients (21%) SD.	[142]
UMIN 000002771	Multiple types (colon, pancreatic, rectal, lung, cervical cancer, epitheloid cancer and papilla cancer)	WT-1, GM-CSF (100 µg), Montanide® ISA-51, ±CpG7909 (100 µg) n = 28 in 2 groups 8 × weekly	ID	I	01/10–11/10	Clinical response: 60% of patients treated with WT-1 + Montanide® ISA-51 + CpG 7909 showed SD, 40% PD. (WT-1 only: 20% SD, 80% PD; WT1 + GM-CSF: 25% SD, 75% PD)	[118]
NCT00669292	Esophageal cancer	URLC10-117 peptide (1 mg), TTK-567 peptide (1 mg), Montanide® ISA-51, + CpG 7909 (0.02, 0.1 mg/kg) n = 9 in 3 groups days: 1, 8, 15, 22	SQ	I/II	11/06–11/09	Serum levels of IFN-α and 2–5 AS increased with increasing dose of CpG 7909. Increase in IFN-α producing Ag-specific CD8 T cell responses in 33% (TTK-567) or 66% (LY6K-177) of the patients. Clinical response: no partial or complete responses, 33% of patients received 0.02 mg/kg CpG 7909 and 66% of patients received 0.1 mg/kg CpG 7909 showed SD.	[143]
NCT00299728	Prostate cancer	NY-ESO-1 (100 µg), +CpG 7909 (2.5 mg) n = 15 one group 4 × 3 week interval	ID	I	03/06–12/06	9 patients (60%) had NY-ESO-1 specific CD4 T cells by week 7–28 (mean 14) and 6 patients (40%) had NY-ESO-1 specific CD8 T cell by week 10–28 (mean 14). 2 patients (13%) developed anti-CpG Ab (1:25,000, 1:6,400). Clinical response: 2 patients (13%) showed no evidence of disease, 8 patients (53%) showed SD and 3 (20%) PD, but no PR or CR was seen after 4 vaccinations.	[128]