Poly(I:C) as cancer vaccine adjuvant : knocking on the door of medical breakthroughs

Reference:

Ammi Rachid, De Waele Jorrit, Willemen Yannick, van Brussel Ilse, Schrijvers Dorien M., Lion Eva, Smits Evelien.- Poly(I:C) as cancer vaccine adjuvant : knocking on the door of medical breakthroughs

Pharmacology and therapeutics - ISSN 0163-7258 - 146(2015), p. 120-131

DOI: http://dx.doi.org/doi:10.1016/j.pharmthera.2014.09.010

Handle: http://hdl.handle.net/10067/1223690151162165141

Review article

Poly(I:C) as cancer vaccine adjuvant: knocking on the door of medical breakthroughs

Rachid AMMI,[1*] Jorrit DE WAELE,[2*] Yannick WILLEMEN,[3] Ilse VAN BRUSSEL,[1] Dorien M. SCHRIJVERS,[1] Eva LION,[3,4] Evelien L.J. SMITS.[2, 3, 4]

Author affiliations:

[1] Laboratory of Physiopharmacology, University of Antwerp, B-2610 Antwerp, Belgium; [2] Center for Oncological Research, University of Antwerp, B-2610 Antwerp, Belgium; [3] Tumor Immunology Group, Laboratory of Experimental Hematology, Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, B-2650 Edegem, Belgium; [4] Center for Cell Therapy & Regenerative Medicine, Antwerp University Hospital, B-2650 Edegem, Belgium.

* Both of these authors contributed equally to this work.

Corresponding author:

Name	Evelien Smits
Email	evelien.smits@uza.be
Phone	003232652576
Fax	003238214456

Abstract

Although cancer vaccination has yielded promising results in patients, objective response rates are low. The right choice of adjuvant might improve efficacy. Here, we review the biological rationale, as well as preclinical and clinical results of polyinosinic:polycytidylic acid and its derivative poly-ICLC as cancer vaccine adjuvants. These synthetic immunological danger signals enhanced vaccine-induced anti-tumor immune responses and contributed to tumor elimination in animal tumor models and patients. Supported by these results, poly-ICLC-containing cancer vaccines are currently extensively studied in ongoing trials, making it highly plausible that poly-ICLC will be part of future approved cancer immunotherapies.

Keywords

poly(I:C), poly-ICLC, cancer vaccine, adjuvant, TLR

Table of Contents

Abbreviations

APC	Antigen-presenting cells
CTL	Cytotoxic T lymphocyte
DC	Dendritic cells
dsRNA	Double stranded RNA
IFN	Interferon
MDA-5	Melanoma differentiation-associated protein 5
NK	Natural killer
ODN	Oligodeoxynucleotides
OLP	Overlapping long peptides
PD-L1	Programmed death-ligand 1
poly(I:C)	Polyriboinosinic-polyribocytidylic acid
poly-ICLC	Poly(I:C) stabilized with poly-L-lysine and carboxymethylcellulose
RIG-I	Retinoic acid-inducible gene 1
TAA	Tumor-associated antigens
TLR	Toll-like receptor
Treg	Regulatory T cells
TTP	Time to disease progression

1. Introduction

To this date, an effective cure remains an elusive goal in many cancers, due to the complexity of tumor cells and their microenvironment. One of the recently acknowledged hallmarks of cancer is its evasion of immune-mediated destruction (Hanahan & Weinberg, 2011). In active cancer immunotherapy, including cancer vaccination, reversing this evasion is endeavored by modulating the host immune system *in vivo* to provoke an effective anti-tumor response.

Standard components of cancer vaccines are tumor-associated antigens (TAA) and immunostimulatory adjuvants (Coffman, Sher, & Seder, 2010). Unfortunately, aluminium compounds as the most widely used vaccine adjuvants are very effective at promoting humoral immune responses, but fail to induce cell-mediated immunity, which is crucial for tumor elimination (Brewer, 2006). In contrast, pathogen-associated molecular patterns have a high capacity to stimulate cell-mediated immunity and are therefore thoroughly investigated as adjuvants in cancer vaccines. For example, the first FDA licensed adjuvant molecule monophosphoryl lipid A - a modified derivative of lipopolysaccharide - was shown to promote immune response broadening (Dubensky & Reed, 2010) and is now a component of an approved cervical cancer vaccine (Schwarz, 2009). This encourages the investigation of the adjuvant effects of other pathogen-associated molecular patterns, including double stranded RNA (dsRNA).

Polyriboinosinic-polyribocytidylic acid (poly(I:C)) and its derivative poly-ICLC (Hiltonol™, i.e. poly(I:C) stabilized with poly-L-lysine and carboxymethylcellulose) are synthetic mimics of viral dsRNA polymers with similar mechanism of action and are included in the National Cancer Institute's ranking of immunotherapeutic agents with the highest potential to boost cancer immunotherapy outcome (Cheever, 2008).Various strategies to include poly(I:C)/poly-ICLC in cancer vaccination are currently being investigated aiming to maximize the stimulation of anti-tumor immunity. In this review, we discuss the biological

rationale, as well as the preclinical and clinical experience with poly(I:C) and poly-ICLC as cancer vaccine adjuvants.

2. Rationale for the use of poly(I:C) and poly-ICLC as cancer vaccine adjuvant

As a dsRNA analogue, poly(I:C) is a ligand of the endosomal Toll-like receptor (TLR)3 (Cheever, 2008). This receptor is widely expressed both in hematopoietic and non-hematopoietic cells (Hewson, Jardine, Edwards, Laza-Stanca, & Johnston, 2005; Schmidt et al., 2004; Tabiasco et al., 2006) with high expression in conventional dendritic cells (DC) (Reis e Sousa, 2004). Following ligation, TLR3 transmits signals via the Toll/interleukin-1 receptor domain-containing adaptor protein inducing interferon (IFN)-β, resulting in the activation of the transcription factors IFN-regulatory factor (IRF), nuclear factor κ-light chain-enhancer of activated B cells and activator protein 1 (Uematsu & Akira, 2007). This activation can lead to apoptosis, production of cytokines and chemokines and maturation of DC (Uematsu & Akira, 2007). Next to signaling via TLR3, poly(I:C) can also bind to the cytoplasmic receptors melanoma differentiation-associated protein 5 (MDA-5) and retinoic acid-inducible gene 1 (RIG-I), although the latter is generally not activated by poly(I:C) (Pichlmair & Reis e Sousa, 2007). Upon dsRNA recognition MDA-5 interacts with its adaptor mitochondria-associated adaptor molecule and a signaling cascade is initiated, leading to activation of the transcription factors IRF3 and nuclear factor κ-light chain-enhancer of activated B cells and to induction of type I IFN (Hiscott, Nguyen, Arguello, Nakhaei, & Paz, 2006).

Nowadays, it is increasingly being recognized that both innate and adaptive immune cells are important mediators of anti-tumor immunity (Kalinski et al., 2006; Lion, Smits, Berneman, & Van Tendeloo, 2012; Shanker & Marincola, 2011). Favorable effects of poly(I:C) on tumor

cells and immune cells have been shown in different *in vitro* and *in vivo* studies, justifying the choice of poly(I:C)/poly-ICLC as adjuvant in cancer vaccination trials.

2.1 Effects on tumor cells

Several tumor cell types express functional TLR3, MDA-5 and/or RIG-I, enabling poly(I:C) to affect tumor cells directly (Cheng & Xu, 2010). Direct effects of poly(I:C) on tumor cells are inhibition of tumor growth and induction of apoptosis (Cheng & Xu, 2010; Smits et al., 2007). By inducing apoptosis in different tumor cell types, poly(I:C) can also indirectly contribute to anti-tumor responses since TAA become available for uptake by antigen-presenting cells (APC). The anti-proliferative effect of poly(I:C) on certain cancer cells is mediated through modulation of different key cell cycle regulatory proteins, as illustrated in human prostate LCaP cells (Paone et al., 2008). Various apoptosis pathways have been suggested for different tumor types; it can be induced through binding of poly(I:C) to TLR3, MDA-5 or RIG-I and apoptosis induction can be IFN-dependent or -independent (Besch et al., 2009; Nomi, Kodama, & Suzuki, 2010; Paone et al., 2008; Salaun, Coste, Rissoan, Lebecque, & Renno, 2006). For the latter, Paone and colleagues showed in a prostate cancer cell model that protein kinase C alpha was responsible for the pro-apoptotic effect (Paone et al., 2008). Poly(I:C) can also induce apoptosis through downregulation of survivin, which is an inhibitor of apoptosis (Nomi et al., 2010). Other key molecules and pathways involved are reviewed in more depth elsewhere (Zhao et al., 2012). The direct anti-tumor effects as described here classify poly(I:C) as a cytotoxic agent.

2.2 Effects on immune cells

Notwithstanding its direct cytotoxic effects, enhancement of both innate and adaptive immunity is considered to be the principal mechanism by which poly(I:C) produces anti-

tumor activity. Poly(I:C)-mediated stimulation has been described for different immune processes and immune cell types, including DC, natural killer (NK) cells and T cells, all major mediators of anti-tumor immunity. In this regard, *in vitro* studies showed that poly(I:C) is able to efficiently mature and activate DC (Benwell, Hruska, Fritsche, & Lee, 2010; Smits et al., 2007; Verdijk et al., 1999), stimulate T cells (Salem et al., 2009), promote NK cell cytotoxicity (Huang, Zheng, & Qiu, 2013; Lauzon, Mian, MacKenzie, & Ashkar, 2006; Lion, Anguille, Berneman, Smits, & Van Tendeloo, 2011; Schmidt et al., 2004), enhance cross-presentation by DC and stimulate DC-NK cell interactions (Akazawa et al., 2007; Lion et al., 2011; Moretta, Marcenaro, Parolini, Ferlazzo, & Moretta, 2008; Zanoni, Granucci, Foti, & Ricciardi-Castagnoli, 2007). Additionally, poly(I:C) induces secretion of pro-inflammatory cytokines both by tumor cells and different immune cell types (Lion, Smits, Berneman, & Van Tendeloo, 2009; McCartney et al., 2009; Salaun et al., 2006; Smits et al., 2010; Smits et al., 2007). Interestingly, it was shown in mice that amongst other TLR agonists, poly(I:C) is the most effective inducer of type I IFN (Longhi et al., 2009), which are important linkers of innate and adaptive immunity. In this way, poly(I:C) also indirectly contributes to activation of different immune cell types including NK cells and T cells.

The cross-presentation pathway, whereby exogenous antigens are taken up by APC and presented to naive $CD8^+$ T lymphocytes, is important for cytotoxic T lymphocyte (CTL) activation against tumor cells. DC are the principal cross-presenting APC *in vivo* (Pooley, Heath, & Shortman, 2001). Although Frleta and colleagues reported an inhibitory effect of poly(I:C) on cross-presentation by human DC *in vitro* (Frleta et al., 2009), different mouse models showed that poly(I:C) activates DC and stimulates cross-priming *in vivo* (Datta et al., 2003; Durand, Wong, Tough, & Le Bon, 2006; McBride, Hoebe, Georgel, & Janssen, 2006; Schulz et al., 2005). Furthermore, uptake of poly(I:C)-induced apoptotic tumor cells by human DC results in poly(I:C)-dependent DC maturation, secretion of pro-inflammatory

cytokines and an increase in their T helper 1-polarizing capacity *in vitro* (Kovalcsik, Lowe, Fischer, Dalgleish, & Bodman-Smith, 2011; Smits et al., 2007), which is in favor of efficient anti-tumor immunity. In addition to CTL, NK cells are increasingly being recognized as immune cells with an important role in anti-tumor immunity. Although it has been shown that poly(I:C) can activate NK cells directly (Akazawa et al., 2007; Huang et al., 2013; Lauzon et al., 2006; McCartney et al., 2009; Pisegna et al., 2004; Schmidt et al., 2004; Sivori et al., 2004), Sivori and colleagues showed that the presence of pro-inflammatory cytokines, such as IL-12 or IL-8, improved NK cell activation, as significant upregulation of NK cytolytic activity was observed (Sivori et al., 2004). In turn, NK cells can contribute to better T cell responses by secreting cytokines in response to poly(I:C) (Salem, El-Naggar, Kadima, Gillanders, & Cole, 2006). In the context of tumor cell vaccination, poly(I:C)-electroporated leukemic or ovarian tumor cells were shown to become more immunogenic and potent IFN-α producers, resulting in activation of DC and NK cells *in vitro* (Kubler et al., 2011; Lion et al., 2011; Lion et al., 2009; Smits et al., 2007). In conclusion, poly(I:C) is able to directly and indirectly activate different immune cell types, including DC, NK cells and T cells, all three important players of the anti-tumor immune response.

3. Preclinical results of poly(I:C)/poly-ICLC as cancer vaccine adjuvant

Several preclinical and clinical studies have been conducted to determine the safety and efficacy of poly(I:C)/poly-ICLC administration as cancer vaccine adjuvant (Table 1-3).

As shown in Table 1, the addition of poly(I:C)/poly-ICLC as single adjuvant to the different antigen formulations, i.e. cell-based, peptide or protein, exosome, or viral vaccines, resulted in enhanced induction of TAA-specific and functional T cells (in terms of IFN-γ production and/or tumor cell lysis). Remarkably, poly(I:C)/poly-ICLC-containing vaccines reduced tumor growth in all studies, independent of the administration route (Table 1). Nevertheless,

the most striking tumor regressions or rejections were observed after intra- or peritumoral poly(I:C) injection (Aranda et al., 2011; Llopiz et al., 2008; Tormo et al., 2006) (Table 2). Also, the effect of poly(I:C) was more pronounced when the cells were transfected with poly(I:C) compared to free administration, as observed in two studies using cellular TAA sources (Cui & Qiu, 2006; McBride et al., 2006), showing that adjuvant efficacy of poly(I:C) can vary depending on the way of administration.

The effect of poly(I:C)/poly-ICLC was further investigated in combination with other adjuvants, such as CpG oligodeoxynucleotides (ODN) and an agonistic anti-CD40 antibody (Table 2). The combined adjuvants were added to viral TAA carriers, peptides or DC-tumor cell fusion hybrids and also resulted in enhanced TAA-specific T cell responses and delayed tumor growth in all studies. However, tumor rejection or eradication was almost exclusively observed when supplementing poly(I:C)/poly-ICLC with CpG ODN or anti-CD40 antibodies (Table 2). Two studies which examined the effect of combining poly(I:C) and CpG ODN as vaccine adjuvants in a lung (Cui & Qiu, 2006) and prostate cancer model (Babiarova et al., 2012) failed to show superiority of the combination over single adjuvant use, supporting further research towards optimal adjuvant combinations.

In the immunological analysis of all studies including poly(I:C)/poly-ICLC as cancer vaccine adjuvant (Table 1 and 2), 20 studies reported an increase in functional antigen-specific $CD8^+$ T cells. Three of these studies showed that the reduced tumor growth was dependent on $CD8^+$ T cells (H. I. Cho & Celis, 2009; Llopiz et al., 2008; Park, Kim, Park, Kim, & Kim, 2011), of which two showed no involvement of $CD4^+$ T cells. Only five studies monitored NK cells in addition to T cells (Aranda et al., 2011; H. I. Cho & Celis, 2009; Llopiz et al., 2008; Park et al., 2011; Wang et al., 2012) (Table 1-2) providing contrasting data regarding the involvement of NK cells in reduction of tumor growth, with two studies showing dependency on NK cells (Llopiz et al., 2008; Park et al., 2011) versus one where reduction was independent of NK cell

presence (H. I. Cho & Celis, 2009). Other findings were an increase in the number of tumor-infiltrating NK cells (and T cells) in a hepatocellular carcinoma model (Wang et al., 2012) and an increase in IFN-γ-producing $CD69^+$ NK cells in a lymphoma model (Aranda et al., 2011). These results point to the need to incorporate NK cells in future immunomonitoring protocols of poly(I:C)-containing vaccines in order to fully unravel its working mechanism.

In summary, preclinical studies demonstrated that poly(I:C) and poly-ICLC are promising adjuvants able to enhance vaccine-induced anti-tumor T cell and NK cell responses. Furthermore, the addition of poly(I:C)/poly-ICLC to cancer vaccines can improve tumor elimination and thus contributes to rejection, regression or eradication of tumors.

4. Clinical experience with poly-ICLC

Early clinical studies using poly(I:C) as stand-alone agent to treat cancer date back to the seventies, but failed to present a beneficial effect of poly(I:C) on clinical outcome (Feldman, Hughes, Darlington, & Kim, 1975; Herr, Kemeny, Yagoda, & Whitmore, 1978; Robinson et al., 1976), probably due to its short half-life (< 30 minutes) (Levy et al., 1975). Its stabilized form poly-ICLC proved to be 5- to 10-fold more resistant to hydrolysis in primate serum and induced significant serum levels of IFN (Levine, Sivulich, Wiernik, & Levy, 1979; Levy et al., 1975). However, the increased stability led to elevated toxicities, including fever, hypotension, arthralgia-myalgia and decreased blood cell counts (Champney, Levine, Levy, & Lerner, 1979; Lampkin, Levine, Levy, Krivit, & Hammond, 1985; Levine et al., 1979). Nevertheless, toxic effects of poly-ICLC could be alleviated by lowering intravenous doses or by injecting intramuscularly (Ewel et al., 1992; Levine et al., 1979; Maluish et al., 1985; O. Nakamura, Shitara, Matsutani, Takakura, & Machida, 1982; Salazar et al., 1996). A pilot study by Salazar and colleagues demonstrated safety and efficacy of long term low dose

intramuscular poly-ICLC administration in malignant glioma patients (Salazar et al., 1996), paving the way for further examination of poly-ICLC's anti-tumor effects in clinical studies.

In contrast to poly-ICLC as stand-alone treatment, its clinical evaluation as cancer vaccine adjuvant was initiated only in the last decade, resulting in ten published studies until now (Table 3), while 29 registered others are ongoing (Supplementary Table 1). Half of the published trials with published results recruited glioma patients (Okada et al., 2012; Okada et al., 2011; Pollack, Jakacki, Butterfield, & Okada, 2012; Prins et al., 2011), whereas a broader array of tumor types is covered in the clinical studies that are currently running (Supplementary Table 1). Poly-ICLC has been and continues to be primarily investigated as adjuvant in peptide or DC vaccination programs, with antibody-containing fusion protein vaccines as the odd one out (Morse et al., 2011) (Table 3 and Supplementary Table 1). Three out of ten trials were conducted with poly-ICLC as the sole adjuvant (Kimura et al., 2013; Okada et al., 2011; Prins et al., 2011), while in the remaining seven poly-ICLC was accompanied by at least one other immune stimulator, i.e. Montanide-ISA-51, Montanide-ISA-51 VG, granulocyte-macrophage colony-stimulating factor or the TLR7/8 agonist resiquimod (Dhodapkar et al., 2014; Morse et al., 2011; Okada et al., 2012; Pollack et al., 2012; Sabbatini et al., 2012).

4.1 Safety and administration

In the published clinical studies (Table 3), poly-ICLC was administered either intramuscularly or subcutaneously in the glioma versus non-glioma trials, respectively, except for one non-glioma study which used both administration routes depending on the entry site of the vaccine (Morse et al., 2011). Vaccination was considered safe and well tolerated in all studies with no difference between administration of poly-ICLC as only adjuvant or as part of an adjuvant combination (Table 3). All studies described local and low-grade injection site reactions upon

addition of poly-ICLC to the vaccine. Six out of nine reports showed adverse events up to grade 2 (Dhodapkar et al., 2014; Kimura et al., 2013; Okada et al., 2012; Prins et al., 2011; Sabbatini et al., 2012) and one study detected only grade 1 events (Kimura et al., 2013), possibly due to its patient population which had a predisposition to - but no advanced - cancer. Grade 3 adverse events (fever, weakness or abdominal pain) were observed in three out of 154 cancer patients that were treated with a poly-ICLC-containing vaccine (Morse et al., 2011; Okada et al., 2012). Although the peptide vaccination regimen of Pollack and colleagues was well tolerated without grade 3 adverse events, they detected pseudoprogression in 25% of glioma patients, which might be due to immune infiltration and is difficult to distinguish from true progression (Pollack et al., 2012). Of the two DC vaccination protocols, Prins and colleagues were able to compare toxicity of the DC vaccine with and without poly-ICLC, detecting no additional toxicity of poly-ICLC when administered concomitantly to antigen-loaded DC (Prins et al., 2011). In conclusion, all vaccinations with poly-ICLC as adjuvant were safe and well tolerated.

4.2 Immunological effects

Given that all but one of the published trials were pilot or phase I studies, no conclusive statements can be drawn beyond the aforementioned safety. Nevertheless, all trials detected immunological responses, including cellular responses, specific for the introduced tumor antigens (Table 3). Three therapeutic studies investigated antigen-specific humoral responses following poly-ICLC adjuvanted vaccination and observed that these were induced or strengthened in 76-92% of the patients compared to 0-62% when poly-ICLC was omitted (Dhodapkar et al., 2014; Morse et al., 2011; Sabbatini et al., 2012). Moreover, addition of poly-ICLC invigorated, fastened en broadened the antibody response (Dhodapkar et al., 2014; Morse et al., 2011; Sabbatini et al., 2012). In a prophylactic setting, 17/39 patients showed a

specific antibody response, of which 12/16 established immune memory, as evidenced by booster vaccination (Kimura et al., 2013). The non-responders presented augmented blood levels of myeloid-derived suppressor cells, which decreased effector T-cell function (Kimura et al., 2013). However, regulatory T cells (Treg) were not elevated, confirming earlier clinical reports (Kimura et al., 2013; Prins et al., 2011; Sabbatini et al., 2012) as well as the preclinical *in vivo* observation that poly(I:C) in its adjuvanting role preferentially promotes antigen-specific effector T cells over Tregs (Perret et al., 2013).

Concomitant poly-ICLC administration stimulated Th1 cytokines, up to a log-fold increase (Okada et al., 2012; Okada et al., 2011; Prins et al., 2011; Tsuji et al., 2013), while Th2 cytokines were either undetectable or reduced (Okada et al., 2012; Tsuji et al., 2013). These elevated Th1 and lowered Th2 cytokines resulted in an increased Th1/Th2 ratio (Prins et al., 2011; Tsuji et al., 2013). Hence, addition of poly-ICLC to cancer vaccines drives T cells towards a Th1 response, which is favored in antitumor immunity (Ikeda et al., 2004). Interestingly, an often used adjuvant, Montanide-ISA-51, drove Th2 polarization, however, this effect was reversed when poly-ICLC is co-administered, as was any Th9 differentiation (Tsuji et al., 2013).

Poly-ICLC adjuvanted vaccines also generated specific cellular responses (Morse et al., 2011; Okada et al., 2011; Pollack et al., 2012; Sabbatini et al., 2012), as observed in 85% of patients by Pollack and colleagues, while 74% of patients showed a $CD8^+$ T cell response in Okada's trial (Okada et al., 2011; Pollack et al., 2012). Furthermore, two studies with control cohorts evidenced the effect of poly-ICLC (Morse et al., 2011; Sabbatini et al., 2012). Morse and colleagues detected specific T cell responses only when the vaccine was adjuvanted by a TLR agonist, including poly-ICLC (Morse et al., 2011). However, Sabbatini and colleagues observed functional T cell responses also in patients treated without poly-ICLC-containing vaccines, albeit much less frequently. $CD8^+$ T cell responses were weak and transient or

sporadic in 25-62% of patients in non-poly-ICLC groups, however, 91% of patients in the poly-ICLC-vaccine cohort showed functional $CD8^+$ T-cell responses, which sustained robustly in 64%. Similarly, $CD4^+$ T cell responses were the strongest in patients treated with poly-ICLC-containing vaccines, in which polyclonality and increased epitope spreading was observed along (Sabbatini et al., 2012). This concurrently led to expansion of high-avidity antigen-specific $CD4^+$ T cells (Tsuji et al., 2013). Remarkably, DC nor NK cells were investigated in any of the clinical trials.

Overall, the applied vaccination strategies adjuvanted by poly-ICLC resulted in objective immunological responses. The contribution of poly-ICLC comprised the enforcement of this immunity, as evidenced by the study of Sabbatini (Sabbatini et al., 2012). The generation of an integrated immune response, consisting of functional $CD8^+$ and $CD4^+$ T cell as well as specific antibody responses, was raised from only 25-31% to 91% upon inclusion of poly-ICLC to the cancer vaccine. Moreover, the immune response was more consistent, faster and broader in this latter group (Sabbatini et al., 2012).

4.3 Clinical effects

As for the immunological data, the design of early phase trials limits the ability to draw conclusive statements about clinical efficacy. Interesting preliminary observations have been described, however: stable disease or regression (minor or complete) was reported in six studies for 87% (Pollack et al., 2012), 59% (Okada et al., 2012) and 50% (Okada et al., 2011) of glioma patients and 25% (Dhodapkar et al., 2014), 18% (Sabbatini et al., 2012) and 3% (Morse et al., 2011) of non-glioma patients treated with a poly-ICLC-containing cancer vaccine (Table 3). The divergent response rates between these two patient populations might be attributed to the tumor types, since other early phase studies also support objective and sometimes durable responses following glioma immunotherapy (Marsh, Goldfarb, Shafman,

& Diaz, 2013). However, the patient populations varied also in their design. The three non-glioma studies recruited patients with advanced malignancies which were either in 2nd or 3rd remission, non-responsive to standard treatment and/or with metastases (Dhodapkar et al., 2014; Morse et al., 2011; Sabbatini et al., 2012). On the other hand, the three glioma studies consisted of mixed patient populations including newly diagnosed and recurrent, low and high grade, treated and non-treated as well as stable disease patients (Okada et al., 2012; Okada et al., 2011; Pollack et al., 2012). Also, the non-glioma patients are on average more than ten years older than the glioma patients, even when the pediatric glioma trial is excluded from this equation. Another striking disparity was the vaccine composition, as glioma patients were treated with peptide or DC vaccines which comprised multiple TAA (Okada et al., 2012; Okada et al., 2011; Pollack et al., 2012), whereas non-glioma patients were treated with peptide or protein vaccines containing one or more epitopes of a single TAA (Morse et al., 2011; Sabbatini et al., 2012). Although preliminary, these data suggest that targeting more than one TAA might enhance the clinical efficacy of immunotherapeutic cancer vaccines and that such approach might be interesting for glioma patients in particular.

The study of Sabbatini (Sabbatini et al., 2012) described an antigen-specific efficacy of their overlapping long peptides (OLP) vaccination approach, since NY-ESO-1^{+} ovarian cancer patients showed a significant increased time to disease progression (TTP) after administration of NY-ESO-1 OLP adjuvanted with poly-ICLC, whereas TTP was unaltered for NY-ESO-1^{-} patients, pointing to the need for proper antigen and patient selection in cancer vaccination study designs. Prins and colleagues, who showed an increased survival in glioblastoma multiforme patients with a mesenchymal gene expression signature in their DC vaccine, stated that addition of poly-ICLC to the DC vaccine improved overall survival compared to historical survival data of sole DC vaccination (Prins et al., 2011). In conclusion, based on

these results, the use of poly-ICLC as an adjuvant shows potential to enhance the clinical efficacy of cancer vaccines.

5. Discussion

As an adjuvant, poly(I:C) is able to target tumor cells and immune cells directly and to activate immune cells and processes indirectly. Preclinical studies using poly(I:C)/poly-ICLC as cancer vaccine adjuvant showed promising results, both alone or when combined with other adjuvants (Tables 1 and 2). Compared to the vaccine counterparts without poly(I:C)/poly-ICLC, addition of the dsRNA molecule resulted in improvement of tumor-specific immune responses as well as in better tumor elimination.

Despite its immunostimulatory capacity, one study showed an inhibitory effect of poly(I:C) on cross-presentation (Frleta et al., 2009). This might have been due to upregulation of inhibiting regulatory molecules, since poly(I:C) upregulated the inhibitory molecule B7-H1 or programmed death-ligand 1 (PD-L1) on DC in a mouse model, which limited $CD8^+$ T cell expansion (Pulko et al., 2009). When combined with a tumor vaccine, selective blocking of PD-L1 increased protection against newly established tumors (Pulko et al., 2009). Hence, additional selective blocking of inhibitory molecules might be able to improve the adjuvant effect of poly(I:C)/poly-ICLC in cancer vaccination strategies. Further improvement might also be made by combined administration of different danger signals, since this has been shown to enhance the activation and Th1-polarizing capacity of human and mice DC *in vitro* (Napolitani, Rinaldi, Bertoni, Sallusto, & Lanzavecchia, 2005). A TLR agonist that was frequently combined with poly(I:C) in preclinical studies is the TLR9 agonist CpG ODN (Table 2). Zheng and colleagues found that the adjuvant effect of CpG with poly(I:C) was required to obtain significant tumor regression in their mouse tumor model (Zheng et al., 2008), demonstrating that TLR agonist combinations are worthy of being tested as combined adjuvants in cancer vaccination trials.

In the ten early phase clinical trials that have been reported using poly-ICLC as cancer vaccine adjuvant, both immunological and clinical activity were reported (Table 3). Although results of these first trials are very promising, two major drawbacks need to be addressed in future studies. First, the lack of controlled studies hampers the allocation of observed effects to certain therapeutic components, making the efficacy evaluation of such components an arduous task. Only four studies contained cohorts without poly-ICLC adjuvant and only three of those reported the results in an adequate separate manner, enabling evaluation of the adjuvant effect. Here, despite an elevated immunological response (Dhodapkar et al., 2014; Morse et al., 2011; Sabbatini et al., 2012), a significant clinical benefit due to the addition of poly-ICLC was limited to a delayed TTP in an antigen specific manner (Sabbatini et al., 2012). Nevertheless, increased OS with poly-ICLC compared to historical DC vaccine only studies was suggested (Prins et al., 2011). Second, research on poly-ICLC as a cancer vaccine adjuvant has only just begun. Following the establishment of the safety of poly-ICLC-containing cancer vaccines as discussed here, it is important to proceed to phase II and III trials. Of the currently registered 29 ongoing studies (www.clinicaltrials.gov), 12 trials include phase II (Supplementary table 1).

In conclusion, poly(I:C) and poly-ICLC effectively contribute to host anti-tumor responses as immunostimulatory components of cancer vaccines. Combining poly(I:C)/poly-ICLC with agents that block immune inhibitory molecules or with other danger signals will probably result in further improvement of cancer vaccine outcome. Although long term effectiveness has not been reported yet, the observed clinical and immunological activity in cancer patients makes poly-ICLC a good adjuvant candidate for cancer vaccines.

References

Akazawa, T., Ebihara, T., Okuno, M., Okuda, Y., Shingai, M., Tsujimura, K., et al. (2007). Antitumor NK activation induced by the Toll-like receptor 3-TICAM-1 (TRIF) pathway in myeloid dendritic cells. [Research Support, Non-U.S. Gov't]. *Proc Natl Acad Sci U S A, 104*(1), 252-257.

Aranda, F., Llopiz, D., Diaz-Valdes, N., Riezu-Boj, J. I., Bezunartea, J., Ruiz, M., et al. (2011). Adjuvant combination and antigen targeting as a strategy to induce polyfunctional and high-avidity T-cell responses against poorly immunogenic tumors. *Cancer Res, 71*(9), 3214-3224.

Babiarova, K., Kutinova, L., Zurkova, K., Krystofova, J., Brabcova, E., Hainz, P., et al. (2012). Immunization with WT1-derived peptides by tattooing inhibits the growth of TRAMP-C2 prostate tumor in mice. [Research Support, Non-U.S. Gov't]. *J Immunother, 35*(6), 478-487.

Banz, A., Cremel, M., Mouvant, A., Guerin, N., Horand, F., & Godfrin, Y. (2012). Tumor growth control using red blood cells as the antigen delivery system and poly(I:C). *J Immunother, 35*(5), 409-417.

Benwell, R. K., Hruska, J. E., Fritsche, K. L., & Lee, D. R. (2010). Double stranded RNA- relative to other TLR ligand-activated dendritic cells induce extremely polarized human Th1 responses. [Research Support, Non-U.S. Gov't]. *Cell Immunol, 264*(2), 119-126.

Besch, R., Poeck, H., Hohenauer, T., Senft, D., Hacker, G., Berking, C., et al. (2009). Proapoptotic signaling induced by RIG-I and MDA-5 results in type I interferon-independent apoptosis in human melanoma cells. [Research Support, Non-U.S. Gov't]. *J Clin Invest, 119*(8), 2399-2411.

Brewer, J. M. (2006). (How) do aluminium adjuvants work? [Review]. *Immunol Lett, 102*(1), 10-15.

Champney, K. J., Levine, D. P., Levy, H. B., & Lerner, A. M. (1979). Modified polyriboinosinic-polyribocytidylic acid complex: sustained interferonemia and its physiological associates in humans. [Research Support, U.S. Gov't, Non-P.H.S.

Research Support, U.S. Gov't, P.H.S.]. *Infect Immun, 25*(3), 831-837.

Cheever, M. A. (2008). Twelve immunotherapy drugs that could cure cancers. *Immunol Rev, 222*, 357-368.

Chen, S., Ou, R., Tang, J., Deng, X., Wu, Y., van Velkinburgh, J. C., et al. (2013). Enhanced anti-tumor effects of HPV16E7(49-57)-based vaccine by combined immunization with poly(I:C) and oxygen-regulated protein 150. *Cancer Epidemiol, 37*(2), 172-178.

Cheng, Y. S., & Xu, F. (2010). Anticancer function of polyinosinic-polycytidylic acid. *Cancer Biol Ther, 10*(12), 1219-1223.

Cho, E. I., Tan, C., Koski, G. K., Cohen, P. A., Shu, S., & Lee, W. T. (2010). Toll-like receptor agonists as third signals for dendritic cell-tumor fusion vaccines. *Head Neck, 32*(6), 700-707.

Cho, H. I., Barrios, K., Lee, Y. R., Linowski, A. K., & Celis, E. (2013). BiVax: a peptide/poly-IC subunit vaccine that mimics an acute infection elicits vast and effective anti-tumor CD8 T-cell responses. *Cancer Immunol Immunother, 62*(4), 787-799.

Cho, H. I., & Celis, E. (2009). Optimized peptide vaccines eliciting extensive CD8 T-cell responses with therapeutic antitumor effects. [Research Support, N.I.H., Extramural

Research Support, Non-U.S. Gov't]. *Cancer Res, 69*(23), 9012-9019.

Coffman, R. L., Sher, A., & Seder, R. A. (2010). Vaccine adjuvants: putting innate immunity to work. *Immunity, 33*(4), 492-503.

Cui, Z., Le, U. M., Qiu, F., & Shaker, D. S. (2007). Learning from viruses: the necrotic bodies of tumor cells with intracellular synthetic dsRNA induced strong anti-tumor immune responses. *Pharm Res, 24*(9), 1645-1652.

Cui, Z., & Qiu, F. (2006). Synthetic double-stranded RNA poly(I:C) as a potent peptide vaccine adjuvant: therapeutic activity against human cervical cancer in a rodent model. *Cancer Immunol Immunother, 55*(10), 1267-1279.

Datta, S. K., Redecke, V., Prilliman, K. R., Takabayashi, K., Corr, M., Tallant, T., et al. (2003). A subset of Toll-like receptor ligands induces cross-presentation by bone marrow-derived dendritic cells. *J Immunol, 170*(8), 4102-4110.

Dhodapkar, M. V., Sznol, M., Zhao, B., Wang, D., Carvajal, R. D., Keohan, M. L., et al. (2014). Induction of antigen-specific immunity with a vaccine targeting NY-ESO-1 to the dendritic cell receptor DEC-205. [Research Support, Non-U.S. Gov't]. *Sci Transl Med, 6*(232), 232ra251.

Domingos-Pereira, S., Decrausaz, L., Derre, L., Bobst, M., Romero, P., Schiller, J. T., et al. (2013). Intravaginal TLR agonists increase local vaccine-specific CD8 T cells and human papillomavirus-associated genital-tumor regression in mice. *Mucosal Immunol, 6*(2), 393-404.

Dubensky, T. W., Jr., & Reed, S. G. (2010). Adjuvants for cancer vaccines. [Review]. *Semin Immunol, 22*(3), 155-161.

Durand, V., Wong, S. Y., Tough, D. F., & Le Bon, A. (2006). IFN-alpha/beta-dependent cross-priming induced by specific toll-like receptor agonists. *Vaccine, 24 Suppl 2*, S2-22-23.

Ewel, C. H., Urba, W. J., Kopp, W. C., Smith, J. W., 2nd, Steis, R. G., Rossio, J. L., et al. (1992). Polyinosinic-polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose in combination with interleukin 2 in patients with cancer: clinical and immunological effects. *Cancer Res, 52*(11), 3005-3010.

Feldman, S., Hughes, W. T., Darlington, R. W., & Kim, H. K. (1975). Evaluation of topical polyinosinic acid-polycytidylic acid in treatment of localized herpes zoster in children with cancer: a randomized, double-blind controlled study. [Clinical Trial

Randomized Controlled Trial

Research Support, U.S. Gov't, P.H.S.]. *Antimicrob Agents Chemother, 8*(3), 289-294.

Frleta, D., Yu, C. I., Klechevsky, E., Flamar, A. L., Zurawski, G., Banchereau, J., et al. (2009). Influenza virus and poly(I:C) inhibit MHC class I-restricted presentation of cell-associated antigens derived from infected dead cells captured by human dendritic cells. *J Immunol, 182*(5), 2766-2776.

Guo, F., Chang, C. K., Fan, H. H., Nie, X. X., Ren, Y. N., Liu, Y. Y., et al. (2008). Anti-tumour effects of exosomes in combination with cyclophosphamide and polyinosinic-polycytidylic acid. *J Int Med Res, 36*(6), 1342-1353.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell, 144*(5), 646-674.

Hansen, J., Lindenstrom, T., Lindberg-Levin, J., Aagaard, C., Andersen, P., & Agger, E. M. (2012). CAF05: cationic liposomes that incorporate synthetic cord factor and poly(I:C) induce CTL immunity and reduce tumor burden in mice. *Cancer Immunol Immunother, 61*(6), 893-903.

Herr, H. W., Kemeny, N., Yagoda, A., & Whitmore, W. F., Jr. (1978). Poly I:C immunotherapy in patients with papillomas or superficial carcinomas of the bladder. [Clinical Trial

Comparative Study

Randomized Controlled Trial

Research Support, U.S. Gov't, Non-P.H.S.

Research Support, U.S. Gov't, P.H.S.]. *Natl Cancer Inst Monogr*(49), 325.

Hewson, C. A., Jardine, A., Edwards, M. R., Laza-Stanca, V., & Johnston, S. L. (2005). Toll-like receptor 3 is induced by and mediates antiviral activity against rhinovirus infection of human bronchial epithelial cells. *J Virol, 79*(19), 12273-12279.

Hiscott, J., Nguyen, T. L., Arguello, M., Nakhaei, P., & Paz, S. (2006). Manipulation of the nuclear factor-kappaB pathway and the innate immune response by viruses. *Oncogene, 25*(51), 6844-6867.

Huang, Y. K., Zheng, Z., & Qiu, F. (2013). Polyinosinic-cytidylic acid as an adjuvant on natural killer- and dendritic cell-mediated antitumor activities. *Tumour Biol, 34*(3), 1615-1623.

Ikeda, H., Chamoto, K., Tsuji, T., Suzuki, Y., Wakita, D., Takeshima, T., et al. (2004). The critical role of type-1 innate and acquired immunity in tumor immunotherapy. [Review]. *Cancer Sci, 95*(9), 697-703.

Kalinski, P., Nakamura, Y., Watchmaker, P., Giermasz, A., Muthuswamy, R., & Mailliard, R. B. (2006). Helper roles of NK and CD8+ T cells in the induction of tumor immunity. Polarized dendritic cells as cancer vaccines. *Immunol Res, 36*(1-3), 137-146.

Kimura, T., McKolanis, J. R., Dzubinski, L. A., Islam, K., Potter, D. M., Salazar, A. M., et al. (2013). MUC1 vaccine for individuals with advanced adenoma of the colon: a cancer immunoprevention feasibility study. *Cancer Prev Res (Phila), 6*(1), 18-26.

Kovalcsik, E., Lowe, K., Fischer, M., Dalgleish, A., & Bodman-Smith, M. D. (2011). Poly(I:C)-induced tumour cell death leads to DC maturation and Th1 activation. *Cancer Immunol Immunother, 60*(11), 1609-1624.

Kubler, K., tho Pesch, C., Gehrke, N., Riemann, S., Dassler, J., Coch, C., et al. (2011). Immunogenic cell death of human ovarian cancer cells induced by cytosolic poly(I:C) leads to myeloid cell maturation and activates NK cells. *Eur J Immunol, 41*(10), 3028-3039.

Lampkin, B. C., Levine, A. S., Levy, H., Krivit, W., & Hammond, D. (1985). Phase II trial of a complex polyriboinosinic-polyribocytidylic acid with poly-L-lysine and carboxymethyl cellulose in the treatment of children with acute leukemia and neuroblastoma: a report from the Children's Cancer Study Group. [Research Support, U.S. Gov't, P.H.S.]. *Cancer Res, 45*(11 Pt 2), 5904-5909.

Lauzon, N. M., Mian, F., MacKenzie, R., & Ashkar, A. A. (2006). The direct effects of Toll-like receptor ligands on human NK cell cytokine production and cytotoxicity. *Cell Immunol, 241*(2), 102-112.

Levine, A. S., Sivulich, M., Wiernik, P. H., & Levy, H. B. (1979). Initial clinical trials in cancer patients of polyriboinosinic-polyribocytidylic acid stabilized with poly-L-lysine, in carboxymethylcellulose [poly(ICLC)], a highly effective interferon inducer. *Cancer Res, 39*(5), 1645-1650.

Levy, H. B., Baer, G., Baron, S., Buckler, C. E., Gibbs, C. J., Iadarola, M. J., et al. (1975). A modified polyriboinosinic-polyribocytidylic acid complex that induces interferon in primates. *J Infect Dis, 132*(4), 434-439.

Lion, E., Anguille, S., Berneman, Z. N., Smits, E. L., & Van Tendeloo, V. F. (2011). Poly(I:C) enhances the susceptibility of leukemic cells to NK cell cytotoxicity and phagocytosis by DC. [Research Support, Non-U.S. Gov't]. *PLoS One, 6*(6), e20952.

Lion, E., Smits, E. L., Berneman, Z. N., & Van Tendeloo, V. F. (2009). Acute myeloid leukemic cell lines loaded with synthetic dsRNA trigger IFN-gamma secretion by human NK cells. [Research Support, Non-U.S. Gov't]. *Leuk Res, 33*(4), 539-546.

Lion, E., Smits, E. L., Berneman, Z. N., & Van Tendeloo, V. F. (2012). NK cells: key to success of DC-based cancer vaccines? *Oncologist, 17*(10), 1256-1270.

Llopiz, D., Dotor, J., Zabaleta, A., Lasarte, J. J., Prieto, J., Borras-Cuesta, F., et al. (2008). Combined immunization with adjuvant molecules poly(I:C) and anti-CD40 plus a tumor antigen has potent prophylactic and therapeutic antitumor effects. *Cancer Immunol Immunother, 57*(1), 19-29.

Longhi, M. P., Trumpfheller, C., Idoyaga, J., Caskey, M., Matos, I., Kluger, C., et al. (2009). Dendritic cells require a systemic type I interferon response to mature and induce CD4+ Th1 immunity with poly IC as adjuvant. *J Exp Med, 206*(7), 1589-1602.

Maluish, A. E., Reid, J. W., Crisp, E. A., Overton, W. R., Levy, H., Foon, K. A., et al. (1985). Immunomodulatory effects of poly(I,C)-LC in cancer patients. [In Vitro

Research Support, U.S. Gov't, P.H.S.]. *J Biol Response Mod, 4*(6), 656-663.

Mansilla, C., Berraondo, P., Durantez, M., Martinez, M., Casares, N., Arribillaga, L., et al. (2012). Eradication of large tumors expressing human papillomavirus E7 protein by therapeutic vaccination with E7 fused to the extra domain a from fibronectin. *Int J Cancer, 131*(3), 641-651.

Marsh, J. C., Goldfarb, J., Shafman, T. D., & Diaz, A. Z. (2013). Current status of immunotherapy and gene therapy for high-grade gliomas. *Cancer Control, 20*(1), 43-48.

McBride, S., Hoebe, K., Georgel, P., & Janssen, E. (2006). Cell-associated double-stranded RNA enhances antitumor activity through the production of type I IFN. *J Immunol, 177*(9), 6122-6128.

McCartney, S., Vermi, W., Gilfillan, S., Cella, M., Murphy, T. L., Schreiber, R. D., et al. (2009). Distinct and complementary functions of MDA5 and TLR3 in poly(I:C)-mediated activation of mouse NK cells. [Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't]. *J Exp Med, 206*(13), 2967-2976.

Moretta, A., Marcenaro, E., Parolini, S., Ferlazzo, G., & Moretta, L. (2008). NK cells at the interface between innate and adaptive immunity. [Review]. *Cell Death Differ, 15*(2), 226-233.

Morse, M. A., Chapman, R., Powderly, J., Blackwell, K., Keler, T., Green, J., et al. (2011). Phase I study utilizing a novel antigen-presenting cell-targeted vaccine with Toll-like receptor stimulation to induce immunity to self-antigens in cancer patients. *Clin Cancer Res, 17*(14), 4844-4853.

Nakamura, O., Shitara, N., Matsutani, M., Takakura, K., & Machida, H. (1982). Phase I-II trials of poly(ICLC) in malignant brain tumor patients. *J Interferon Res, 2*(1), 1-4.

Nakamura, T., Moriguchi, R., Kogure, K., & Harashima, H. (2013). Incorporation of polyinosine-polycytidylic acid enhances cytotoxic T cell activity and antitumor effects by octaarginine-modified liposomes encapsulating antigen, but not by octaarginine-modified antigen complex. *Int J Pharm, 441*(1-2), 476-481.

Napolitani, G., Rinaldi, A., Bertoni, F., Sallusto, F., & Lanzavecchia, A. (2005). Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. *Nat Immunol, 6*(8), 769-776.

Nomi, N., Kodama, S., & Suzuki, M. (2010). Toll-like receptor 3 signaling induces apoptosis in human head and neck cancer via survivin associated pathway. *Oncol Rep, 24*(1), 225-231.

Okada, H., Butterfield, L. H., Sakaki, M., Hoji, A., Salazar, A. M., Shaw, E. G., et al. (2012). *Abstract LB-135: A pilot study of peptide-based vaccines in combination with poly ICLC in patients with WHO grade 2 low-grade glioma*. Paper presented at the American Association for Cancer Research.

Okada, H., Kalinski, P., Ueda, R., Hoji, A., Kohanbash, G., Donegan, T. E., et al. (2011). Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with {alpha}-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. *J Clin Oncol, 29*(3), 330-336.

Paone, A., Starace, D., Galli, R., Padula, F., De Cesaris, P., Filippini, A., et al. (2008). Toll-like receptor 3 triggers apoptosis of human prostate cancer cells through a PKC-alpha-dependent mechanism. *Carcinogenesis, 29*(7), 1334-1342.

Park, J. S., Kim, H. S., Park, H. M., Kim, C. H., & Kim, T. G. (2011). Efficient induction of anti-tumor immunity by a TAT-CEA fusion protein vaccine with poly(I:C) in a murine colorectal tumor model. *Vaccine, 29*(47), 8642-8648.

Parvizi, P., Mallick, A. I., Haq, K., Haghighi, H. R., Orouji, S., Thanthrige-Don, N., et al. (2012). A toll-like receptor 3 ligand enhances protective effects of vaccination against Marek's disease virus and hinders tumor development in chickens. *Viral Immunol, 25*(5), 394-401.

Perret, R., Sierro, S. R., Botelho, N. K., Corgnac, S., Donda, A., & Romero, P. (2013). Adjuvants that improve the ratio of antigen-specific effector to regulatory T cells enhance tumor immunity. [Research Support, Non-U.S. Gov't]. *Cancer Res, 73*(22), 6597-6608.

Pichlmair, A., & Reis e Sousa, C. (2007). Innate recognition of viruses. *Immunity, 27*(3), 370-383.

Pisegna, S., Pirozzi, G., Piccoli, M., Frati, L., Santoni, A., & Palmieri, G. (2004). p38 MAPK activation controls the TLR3-mediated up-regulation of cytotoxicity and cytokine production in human NK cells. *Blood, 104*(13), 4157-4164.

Pollack, I. F., Jakacki, R. I., Butterfield, L. H., & Okada, H. (2012). *Abstract LB-131: Peptide vaccine therapy for childhood gliomas: interim results of a pilot study* Paper presented at the American Association for Cancer Research.

Pooley, J. L., Heath, W. R., & Shortman, K. (2001). Cutting edge: intravenous soluble antigen is presented to CD4 T cells by CD8- dendritic cells, but cross-presented to CD8 T cells by CD8+ dendritic cells. *J Immunol, 166*(9), 5327-5330.

Prins, R. M., Soto, H., Konkankit, V., Odesa, S. K., Eskin, A., Yong, W. H., et al. (2011). Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. *Clin Cancer Res, 17*(6), 1603-1615.

Pulko, V., Liu, X., Krco, C. J., Harris, K. J., Frigola, X., Kwon, E. D., et al. (2009). TLR3-stimulated dendritic cells up-regulate B7-H1 expression and influence the magnitude of CD8 T cell responses to tumor vaccination. *J Immunol, 183*(6), 3634-3641.

Reis e Sousa, C. (2004). Activation of dendritic cells: translating innate into adaptive immunity. *Curr Opin Immunol, 16*(1), 21-25.

Robinson, R. A., DeVita, V. T., Levy, H. B., Baron, S., Hubbard, S. P., & Levine, A. S. (1976). A phase I-II trial of multiple-dose polyriboinosic-polyribocytidylic acid in patieonts with leukemia or solid tumors. *J Natl Cancer Inst, 57*(3), 599-602.

Sabbatini, P., Tsuji, T., Ferran, L., Ritter, E., Sedrak, C., Tuballes, K., et al. (2012). 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, 18*(23), 6497-6508.

Salaun, B., Coste, I., Rissoan, M. C., Lebecque, S. J., & Renno, T. (2006). TLR3 can directly trigger apoptosis in human cancer cells. *J Immunol, 176*(8), 4894-4901.

Salazar, A. M., Levy, H. B., Ondra, S., Kende, M., Scherokman, B., Brown, D., et al. (1996). Long-term treatment of malignant gliomas with intramuscularly administered polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose: an open pilot study. *Neurosurgery, 38*(6), 1096-1103; discussion 1103-1094.

Salem, M. L., Diaz-Montero, C. M., El-Naggar, S. A., Chen, Y., Moussa, O., & Cole, D. J. (2009). The TLR3 agonist poly(I:C) targets CD8+ T cells and augments their antigen-specific responses upon their adoptive transfer into naive recipient mice. *Vaccine, 27*(4), 549-557.

Salem, M. L., El-Naggar, S. A., Kadima, A., Gillanders, W. E., & Cole, D. J. (2006). The adjuvant effects of the toll-like receptor 3 ligand polyinosinic-cytidylic acid poly (I:C) on antigen-specific CD8+ T cell responses are partially dependent on NK cells with the induction of a beneficial cytokine milieu. *Vaccine, 24*(24), 5119-5132.

Salem, M. L., Kadima, A. N., Cole, D. J., & Gillanders, W. E. (2005). Defining the antigen-specific T-cell response to vaccination and poly(I:C)/TLR3 signaling: evidence of enhanced primary and memory CD8 T-cell responses and antitumor immunity. *J Immunother, 28*(3), 220-228.

Schmidt, K. N., Leung, B., Kwong, M., Zarember, K. A., Satyal, S., Navas, T. A., et al. (2004). APC-independent activation of NK cells by the Toll-like receptor 3 agonist double-stranded RNA. *J Immunol, 172*(1), 138-143.

Schulz, O., Diebold, S. S., Chen, M., Naslund, T. I., Nolte, M. A., Alexopoulou, L., et al. (2005). Toll-like receptor 3 promotes cross-priming to virus-infected cells. *Nature, 433*(7028), 887-892.

Schwarz, T. F. (2009). Clinical update of the AS04-adjuvanted human papillomavirus-16/18 cervical cancer vaccine, Cervarix. [Research Support, Non-U.S. Gov't

Review]. *Adv Ther, 26*(11), 983-998.

Shanker, A., & Marincola, F. M. (2011). Cooperativity of adaptive and innate immunity: implications for cancer therapy. *Cancer Immunol Immunother, 60*(8), 1061-1074.

Sivori, S., Falco, M., Della Chiesa, M., Carlomagno, S., Vitale, M., Moretta, L., et al. (2004). CpG and double-stranded RNA trigger human NK cells by Toll-like receptors: induction of cytokine release and cytotoxicity against tumors and dendritic cells. *Proc Natl Acad Sci U S A, 101*(27), 10116-10121.

Smits, E. L., Cools, N., Lion, E., Van Camp, K., Ponsaerts, P., Berneman, Z. N., et al. (2010). The Toll-like receptor 7/8 agonist resiquimod greatly increases the immunostimulatory capacity of human acute myeloid leukemia cells. [Research Support, Non-U.S. Gov't]. *Cancer Immunol Immunother, 59*(1), 35-46.

Smits, E. L., Ponsaerts, P., Van de Velde, A. L., Van Driessche, A., Cools, N., Lenjou, M., et al. (2007). Proinflammatory response of human leukemic cells to dsRNA transfection linked to activation of dendritic cells. [Research Support, Non-U.S. Gov't]. *Leukemia, 21*(8), 1691-1699.

Tabiasco, J., Devevre, E., Rufer, N., Salaun, B., Cerottini, J. C., Speiser, D., et al. (2006). Human effector CD8+ T lymphocytes express TLR3 as a functional coreceptor. *J Immunol, 177*(12), 8708-8713.

Tormo, D., Ferrer, A., Bosch, P., Gaffal, E., Basner-Tschakarjan, E., Wenzel, J., et al. (2006). Therapeutic efficacy of antigen-specific vaccination and toll-like receptor stimulation against established transplanted and autochthonous melanoma in mice. *Cancer Res, 66*(10), 5427-5435.

Tsuji, T., Sabbatini, P., Jungbluth, A. A., Ritter, E., Pan, L., Ritter, G., et al. (2013). Effect of Montanide and poly-ICLC adjuvant on human self/tumor antigen-specific CD4+ T cells in phase I overlapping long peptide vaccine trial. [Research Support, Non-U.S. Gov't]. *Cancer Immunol Res, 1*(5), 340-350.

Uematsu, S., & Akira, S. (2007). Toll-like receptors and Type I interferons. *J Biol Chem, 282*(21), 15319-15323.

Verdijk, R. M., Mutis, T., Esendam, B., Kamp, J., Melief, C. J., Brand, A., et al. (1999). Polyriboinosinic polyribocytidylic acid (poly(I:C)) induces stable maturation of functionally active human dendritic cells. [Research Support, Non-U.S. Gov't]. *J Immunol, 163*(1), 57-61.

Wang, C., Zhuang, Y., Zhang, Y., Luo, Z., Gao, N., Li, P., et al. (2012). 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, 30*(32), 4790-4799.

Wick, D. A., Martin, S. D., Nelson, B. H., & Webb, J. R. (2011). Profound CD8+ T cell immunity elicited by sequential daily immunization with exogenous antigen plus the TLR3 agonist poly(I:C). *Vaccine, 29*(5), 984-993.

Xiao, H., Peng, Y., Hong, Y., Huang, L., Guo, Z. S., Bartlett, D. L., et al. (2013). Local administration of TLR ligands rescues the function of tumor-infiltrating CD8 T cells and enhances the antitumor effect of lentivector immunization. *J Immunol, 190*(11), 5866-5873.

Zanoni, I., Granucci, F., Foti, M., & Ricciardi-Castagnoli, P. (2007). Self-tolerance, dendritic cell (DC)-mediated activation and tissue distribution of natural killer (NK) cells. [Review]. *Immunol Lett, 110*(1), 6-17.

Zhao, X., Ai, M., Guo, Y., Zhou, X., Wang, L., Li, X., et al. (2012). Poly I:C-induced tumor cell apoptosis mediated by pattern-recognition receptors. *Cancer Biother Radiopharm, 27*(9), 530-534.

Zheng, R., Cohen, P. A., Paustian, C. A., Johnson, T. D., Lee, W. T., Shu, S., et al. (2008). Paired Toll-like receptor agonists enhance vaccine therapy through induction of interleukin-12. *Cancer Res, 68*(11), 4045-4049.

Zhu, X., Nishimura, F., Sasaki, K., Fujita, M., Dusak, J. E., Eguchi, J., et al. (2007). Toll like receptor-3 ligand poly-ICLC promotes the efficacy of peripheral vaccinations with tumor antigen-derived peptide epitopes in murine CNS tumor models. *J Transl Med, 5*, 10.

Tables and supplements

Table 1. Preclinical studies on the use of poly(I:C) or poly-ICLC as single adjuvant in cancer vaccines.

Reference	Tumor type (model)	Antigen source (administration route)	Poly(I:C) or poly-ICLC (administration route)	Vaccine results without poly(I:C) or poly-ICLC	Effect of poly(I:C) or poly-ICLC
Cell-based vaccines					
(Schulz et al., 2005)	Not specified (Vero-OVA)	Apoptotic tumor cell (IV/IP)	Poly(I:C) electroporated (IV/IP)	OVA-specific functional CTL *in vivo* Tumor cell killing	↗ cross-priming responses ↗ tumor cell killing
(McBride et al., 2006)	Lymphoma (EL-4-mOVA)	Apoptotic EL-4-mOVA (SC)	Poly(I:C) (IC) Poly(I:C) (co-ad)	OVA-specific functional CTL *in vivo* Tumor cell killing	<u>Poly(I:C) only effective IC:</u> ↗ functional specific CTL ↘ tumor growth, ↗ survival
(Cui, Le, Qiu, & Shaker, 2007)	Lung (TC-1)	Necrotic tumor cell bodies (SC)	Poly(I:C) (IC) Poly(I:C) (co-ad)	No effect on splenocyte proliferation No therapeutic effect	<u>Poly(I:C) (IC) > co-ad:</u> ↗ functional specific CTL ↘ tumor growth, ↗ survival
(Banz et al., 2012)	Lymphoma (E.G7-OVA)	OVA-loaded RBC (IV)	Poly(I:C) (co-ad)	n.d.	<u>Effect of complete vaccine:</u> ↗ specific $CD8^+$ T cells ↘ tumor growth
	Melanoma (B16-F10)	TRP2-loaded RBC (IV)			

Table 1 (continued)

Reference	Tumor type (model)	Antigen source (administration route)	Poly(I:C) or poly-ICLC (administration route)	Vaccine results without poly(I:C) or poly-ICLC	Effect of poly(I:C) or poly-ICLC
Peptide/protein vaccines					
(Salem, Kadima, Cole, & Gillanders, 2005)	Thymoma (E.G7) Melanoma metastatic to lung (B16-OVA)	OVA peptide (SC)	Poly(I:C) (IP)	Transient ↗ specific $CD8^+$ T cells	Sustained ↗ functional specific CTL ↘ tumor growth, tumor rejection
(Park et al., 2011)	Colorectal (MC-38-cea2)	TAT-CEA fusion protein (SC)	Poly(I:C) (co-ad)	No effect	Effect dependent on $CD8^+$ T cells and NK cells, not on $CD4^+$ T cells ↘ tumor growth, partial tumor rejection
(Wick, Martin, Nelson, & Webb, 2011)	Lymphoma (E.G7-OVA)	OVA peptide (SC)	Poly(I:C) (co-ad)	No specific $CD8^+$ T cells Therapeutic outcome n.d.	Functional specific $CD8^+$ T cells Tumor regression , ↗ survival # doses ~ therapeutic effect

Table 1 (continued)

Reference	Tumor type (model)	Antigen source (administration route)	Poly(I:C) or poly-ICLC (administration route)	Vaccine results without poly(I:C) or poly-ICLC	Effect of poly(I:C) or poly-ICLC
(Banz et al., 2012)	Lymphoma (E.G7-OVA) Melanoma (B16-F10)	OVA peptide (IV) TRP2 peptide (IV)	Poly(I:C) (co-ad)	n.d.	Effect of complete vaccine: Low ↗ specific $CD8^+$ T cells > 2 injections: ↘ tumor growth
(Mansilla et al., 2012)	Lung (TC-1)	EDA-HPV16E7 fusion protein (IV)	Poly(I:C) (co-ad)	Induction functional CTL Low ↘ tumor growth	↗ functional specific CTL Tumor eradication, ↗ survival
(Wang et al., 2012)	Hepatocellular carcinoma (Hepa1-6)	Tumor cell lysate in liposomes (IP)	Poly(I:C) (co-ad as free agent) Poly(I:C) (co-ad within liposome)	No effect	Co-ad as free agent: No immunological effect Low ↘ tumor growth Co-ad in liposomes: ↗ functional specific CTL; ↗ number of TIL and tumor-infiltrating NK cells Tumor regression

Table 1 (continued)

Reference	Tumor type (model)	Antigen source (administration route)	Poly(I:C) or poly-ICLC (administration route)	Vaccine results without poly(I:C) or poly-ICLC	Effect of poly(I:C) or poly-ICLC
(Domingos-Pereira et al., 2013)	Genital (TC-1-luc)	HPV16E7 peptide (SC)	Poly(I:C) (IVAG 5 days after peptide vaccination)	Low specific T cell count	↗ $CD4^+$ T cells and functional specific $CD8^+$ T cells in genital mucosa
(T. Nakamura, Moriguchi, Kogure, & Harashima, 2013)	Lymphoma (E.G7-OVA)	OVA-containing liposomes (SC)	Poly(I:C) (co-ad within liposome)	Low CTL activity Therapeutic: no effect Prophylactic: partial tumor rejection	↗↗ CTL activity Therapeutic: ↘ tumor growth Prophylactic: ↗ tumor rejection
(H. I. Cho, Barrios, Lee, Linowski, & Celis, 2013)†	Lung (TC-1)	HPV16-E7 peptide (IV/SC/IM)	Poly-ICLC (co-ad)	n.d.	<u>Effect of complete vaccine:</u> ↗ antigen-specific $CD8^+$ T cells ↘ immune response when poly-ICLC administered prior to vaccine Tumor eradication
	Melanoma (B16-F10)	Pam2-TRP1 peptide (IV/SC/IM)			
(Banz et al., 2012; Perret et al., 2013)	Lymphoma (E.G7) Melanoma (B16)	OVA peptide (SC)	Poly(I:C) (co-ad)	No effect	<u>Effect of complete vaccines:</u> ↗ Tumor rejection and ↗ Survival

Table 1 (continued)

Reference	Tumor type (model)	Antigen source (administration route)	Poly(I:C) or poly-ICLC (administration route)	Vaccine results without poly(I:C) or poly-ICLC	Effect of poly(I:C) or poly-ICLC
Other vaccines					
(Guo et al., 2008)	Lymphocytic leukemia (L1210)	DC-derived exosome (SC)	Poly(I:C) (co-ad)	No effect	↘ tumor growth, ↗ survival
(Parvizi et al., 2012)§	Lymphoma (MDV-RB1B)	HVT (Aerosol)	Poly(I:C) (pre-infection aerosol; post-infection IM)	Minor tumor rejection	Poly(I:C) pre- and post-infection: ↘ IL-10 (Treg marker) Partial tumor protection

Abbreviations: †, poly-ICLC was used; §, study was done in chickens, other studies in mice; Ag, antigen; CEA, carcinoembryonic antigen; co-ad, combined administration of antigen source and adjuvans; CPA, cyclophosphamide; CTL, cytotoxic T lymphocyte; DC, dendritic cell; EDA, extra domain A from fibronectin; HPV16E7, human papilloma virus protein E7; HVT, herpesvirus of turkeys; IC, intracellular; IFN, interferon; IL, interleukin; IM, intramuscular; IP, intraperitoneal; IV, intravenous; IVAG, intravaginal; mAb, monoclonal antibody; MD, Marek's disease; MDV-RB1B, Marek's disease virus strain RB1B; n.d., not determined; OVA, ovalbumin; PD-L1, programmed death-ligand 1; Poly(I:C), polyinosinic:polycytidylic acid; Poly-ICLC, poly(I:C) stabilized with poly L-Lysine and carboxymethylcellulose; RBC, red blood cell; SC, subcutaneous; TAT, transactivator of transcription; Pam2, 2 palmitic acid chains; Treg, regulatory T cell; TRP, tyrosinase-related protein.

Table 2. Preclinical studies on the use of poly(I:C) or poly-ICLC as part of a combination adjuvant in cancer vaccines.

Reference	Tumor (model)	Antigen source (administration route)	Extra adjuvant component	Poly(I:C) or poly-ICLC (administration route)	Vaccine results without poly(I:C) or poly-ICLC	Effect of poly(I:C) or poly-ICLC
In combination with CpG ODN						
(Tormo et al., 2006)	Melanoma (B16 or DMBA/TPA) Metastatic melanoma to lung (B16)	Ad-hTRP2 (IP)	CpG ODN1826	Poly(I:C) later (IT/PT in cutaneous melanoma; ID in melanoma metastatic to lung)	Viral vector only: ↘ tumor growth, ↗ survival	Viral vector + poly(I:C) + CpG: Induction TRP2-specific cellular & humoral immunity ↘↘↘ tumor growth, ↘ metastases, ↗↗ survival
(Cui & Qiu, 2006)	Lung (TC-1)	HPV16-E7$_{49-57}$ peptide (SC)	CpG ODN1826	Poly(I:C) (co-ad complexed to peptide)	Peptide only: no effect Peptide + CpG: ↘ tumor growth	Peptide + poly(I:C): most effective Functional specific CTL ↗ DC in draining LN ↘↘ tumor growth Tumor rejection upon rechallenge
(Zheng et al., 2008)	Fibrosarcoma metastatic to lung (MCA205)	DC-tumor fusion hybrid (IN)	CpG ODN1826	Poly(I:C) concomitant and later (IP)	Fusion hybrid only or fusion hybrid + one TLR ligand: No significant effect on tumor growth and survival	Fusion hybrid + poly(I:C) + CpG: Tumor eradication, ↗ survival ↗ functional specific CD8/CD4 T cells

Table 2 (continued)

Reference	Tumor (model)	Antigen source (administration route)	Extra adjuvant component	Poly(I:C) or poly-ICLC (administration route)	Vaccine results without poly(I:C) or poly-ICLC	Effect of poly(I:C) or poly-ICLC
(E. I. Cho et al., 2010)	Melanoma metastatic to lung (D5LacZ)	DC–tumor fusion hybrids (IN)	CpG ODN1826	Poly(I:C) concomitant and later (IP)	Fusion hybrid only: ↘ metastases	Fusion hybrid + poly(I:C) + CpG: ↘↘ metastases
(Babiarova et al., 2012)	Prostate (TRAMP-C2)	WT1 peptide (SC/tattoo)	CpG ODN1826	Poly(I:C) (IP)	Peptide + CpG: Functional specific CTL No ↘ tumor growth	Peptide + CpG + poly(I:C): ↘ tumor growth (but less compared to only Cpg + poly(I:C))
(Xiao et al., 2013)	Melanoma (B16-F10)	hgp100-lv (SC)	CpG ODN1668	Poly(I:C) later (IT)	Viral vector only: ↘ tumor growth, ↗ survival ↗ IT $CD4^+$, $CD8^+$ and Treg cells Viral vector + one TLR ligand: No significant improvement	Viral vector + poly(I:C) + CpG: Tumor regression, ↗↗ survival ↘ IT $CD4^+$, $CD8^+$ and Treg cells ↗ $CD8^+$/Treg ratio Restoration TIL function

Table 2 (continued)

Reference	Tumor (model)	Antigen source (administration route)	Extra adjuvant component	Poly(I:C) or poly-ICLC (administration route)	Vaccine results without poly(I:C) or poly-ICLC	Effect of poly(I:C) or poly-ICLC
In combination with anti-CD40						
(Llopiz et al., 2008)	Lymphoma (E.G7–OVA)	OVA peptide (IV/IT)	Agonistic anti-CD40 antibody	Poly(I:C) (co-ad)	<u>Peptide only:</u> Low number of functional specific $CD8^+$ T cells ↘ tumor growth, ↗ survival <u>Peptide + single adjuvant:</u> ↘↘ tumor growth, ↗ survival	<u>Peptide + poly(I:C) + anti-CD40:</u> Functional specific $CD8^+$ and $CD4^+$ T cells $CD8^+$ T cells and NK cells involved in tumor eradication Tumor eradication and 100% survival when frequently applied Tumor rejection upon re/challenge, 100% survival
(H. I. Cho & Celis, 2009) †	Melanoma metastatic to lung (B16F10)	TRP2 peptide (IV)	Agonistic anti-CD40 antibody IFA	Poly-ICLC (co-ad)	n.d.	<u>Peptide + poly(I:C) + anti-CD40:</u> Durable functional specific $CD8^+$ T cells $CD8^+$ T cells involved in tumor eradication, not $CD4^+$ T or NK cells

						Tumor eradication, ↗ survival Partial tumor rejection upon tumor challenge, full rejection upon rechallenge
(Aranda et al., 2011)	Melanoma (B16-OVA)	OVA peptide (IT)	Imiquimod and agonistic anti-CD40 antibody	Poly(I:C) (IT)	<u>Peptide + imiquimod + anti-CD40:</u> ↗ activation of NK cells ↗ activation and ↗ DC ↘ tumor growth	<u>Peptide + imiquimod + anti-CD40 + poly(I:C)</u> ↗↗ activation of NK cells (IFN-γ) ↗↗ functional specific CTL ↗↗ activation and ↗↗ DC ↘ tumor growth, ↗ survival Tumor rejection upon rechallenge
In combination with other adjuvantia						
(Zhu et al., 2007) †	Glioma (GL261)	Peptide: mTRP2, hgp100 and mEphA2 (SC)	IFA	Poly-ICLC (IM)	<u>Peptide + IFA:</u> ↗ functional specific CTL ↗ BIL and ↗ survival Partial tumor rejection upon rechallenge	<u>Peptide + IFA + poly(I:C):</u> ↗↗ functional specific CTL ↗↗ BIL and ↗↗ survival ↗ tumor rejection upon rechallenge
	Melanoma (M05)	OVA peptide (SC)				

Table 2 (continued)

Reference	Tumor (model)	Antigen source (administration route)	Extra adjuvant component	Poly(I:C) or poly-ICLC (administration route)	Vaccine results without poly(I:C) or poly-ICLC	Effect of poly(I:C) or poly-ICLC
(Hansen et al., 2012)	Lung (TC-1) Melanoma metastatic to lung (B16-OVA)	Peptide: HPV16E7 (IP) Peptide: OVA and TRP2 (IP)	CAF01 Montanide-ISA-720 IFA	Poly(I:C) (co-ad)	Peptide: Partial tumor rejection upon tumor challenge Therapeutic: no effect Peptide + CAF01: ↗ functional specific CTL ↗ IT specific CTL ↘ metastases upon challenge Therapeutic: no effect	Peptide + poly(I:C): Therapeutic: ↘ tumor growth, ↗ survival Peptide + CAF01 + poly(I:C): ↗↗ functional specific CTL ↗↗ IT specific CTL Full tumor rejection upon tumor challenge Therapeutic: ↘ tumor growth, ↘ metastases, ↗ survival (better than Montanide-ISA-720 + poly(I:C))
(Chen et al., 2013)	Lung (TC-1)	$HPV16E7_{49–57}$ peptide (IP)	ORP150	Poly(I:C) (co-ad)	Peptide: ↗↗ functional specific CTL ↘ tumor growth, ↗ survival Peptide + ORP150:	Peptide + poly(I:C) ↗↗ functional specific CTL ↘↘ tumor growth, ↗↗ survival Peptide + poly(I:C) + ORP150:

		↗↗ functional specific CTL ↘↘ tumor growth, ↗↗ survival	↗↗↗ functional specific CTL ↘↘↘ tumor growth, ↗↗↗ survival

Abbreviations: †, poly-ICLC was used instead of poly(I:C); Ad, adenovirus; Ag, antigen; BIL, brain infiltrating lymphocyte; CAF01, cationic liposomes that incorporate synthetic cord factor and poly(I:C); CNS, central nervous system; combined, combined administration of antigen source and adjuvans; CpG ODN, CpG oligodeoxynucleotides; CTL, cytotoxic T lymphocyte; DC, dendritic cell; DMBA, 7,12-dimethylbenz(α)anthracene; mEphA2, mouse ephrin type-A receptor 2; HBV, hepatitis B virus; hgp100-lv, human glycoprotein 100 lentivirus; HPV16E7, human papilloma virus protein E7; ID, intradermal; IFA, incomplete Freund's Adjuvant; IM, intramuscular; IP, intraperitoneal; IT, intratumoral; IV, intravenous; LN, lymph node; n.d., not determined; NK cells, natural killer cells; ORP150, oxygen-regulated protein 150; OVA, ovalbumin; Poly(I:C), polyinosinic:polycytidylic acid; Poly-ICLC, poly(I:C) stabilized with poly L-Lysine and carboxymethyl cellulose; PT, peritumoral; SC, subcutaneous; Teff, T effector cell; TIL, tumor-infiltrating lymphocyte; Top, topical; TPA, 12-O-tetradecanoylphorbol-13-acetate; Treg, regulatory T cells; (m/h)TRP2, (mouse/human) tyrosinase-related protein 2.

Table 3. Overview of published clinical cancer vaccine immunotherapy trials with poly-ICLC as adjuvant.

Reference	Indication	n	Phase	Antigen source (administration route)	Combined adjuvant	Poly-ICLC administration route	Safety	Immunologcal effects	Clinical effects
Glioma trials									
(Okada et al., 2011) (NCT00766753)	Recurrent HGG	22	I/II	GAA-loaded αDC1 (EphA2, gp100, IL-13Rα2, YKL-40) (IN)	n.a.	IM later	≤ AE2	14/19 functional, GAA-specific $CD8^+$ T cells ↗ type 1 cytokines & chemokines	1 CR, 1 PR, 9 SD
(Prins et al., 2011) (NCT00068510)	Glioblastoma	3/2 3*	I	Autologous tumor lysate-pulsed DC (ID)	n.a.	/	≤ AE2	↗ serum Th1 cytokines, ↗ Th1/Th2 cytokine ratio = Tregs; ↗ $CD8^+$ TIL in Mes group	<u>Total group:</u> ↗ OS with DC vaccine compared to controls in Mes group, =OS in PN group
					n.a.	IM concomitant with boosters at vaccine injection site	≤ AE2	Log-fold ↗ serum Th1 cytokines$^{\Omega}$	

Table 3 (continued)

Reference	Indication	n	Phase	Antigen source (administration route)	Combined adjuvant	Poly-ICLC administration route	Safety	Immunologcal effects	Clinical effects
(Okada et al., 2012) [¥] (NCT00874861) (NCT00795457)	LGG	24	Pilot	GAA peptides (EphA2, IL-13Rα2, survivin, WT1) (SC)	Montanide -ISA-51	IM	1 AE3	Robust, sustained type 1 anti-GAA T-cell responses; absent or transient type 2 responses	10/17 SD after complete treatment
(Pollack et al., 2012) [¥] (NCT01130077)	Pediatric HGG	24	Pilot	GAA peptides (EphA2, IL-13Rα2, surviving) (SC)	Montanide -ISA-51 TT	IM concomitant	≤ AE2, pseudo-progression	11/13 functional GAA-specific responses	1 CR, 3 PR, 1 MR, 16 SD, 3 PD

Table 3 (continued)

Reference	Indication	n	Phase	Antigen source (administration route)	Combined adjuvant	Poly-ICLC administration route	Safety	Immunologcal effects	Clinical effects
Non-glioma trials									
(Morse et al., 2011) (NCT00709462) (NCT00648102)	Advanced epithelial malignancies	31/87*	I	CDX-1307 fusion protein (ManR-ab fused to hCG-β protein) (ID/IV)	ID vaccine: GM-CSF + resiquimod; IV vaccine: GM-CSF	n.a.	3 AE3	hCG-β-specific IgG1 and IgM in 7/50, low titers Functional hCG-β-specific T cells (only with resiquimod)	8/56 SD (2.3-18.2 months)
						ID vaccine: SC concomitant and later; IV vaccine: IM concomitant and later	2 AE3, ↗ injection site reactions	hCG-β-specific IgG1 and IgM in 19/25, ↗ titers Functional hCG-β-specific T cells	1/31 SD (8.8 months)
(Sabbatini et al., 2012) (NCT00616941)	Advanced ovarian cancer in 2nd or 3rd remission	11/28*	I	NY-ESO-1 OLP (SC)	n.a.	n.a.	≤ AE2	No humoral response 1/4 functional $CD8^+$ T cells Functional $CD4^+$ T cells; inhibition of high-avidity $CD4^+$ precursors	1 CR, 3 PD

(Tsuji et al., 2013) (NCT00616941)	4/12*, selected from 28 above	Montanide-ISA-51 VG	n.a.	≤ AE2	NY-ESO-1 specific IgG in 6/13 8/13 weak, transient functional $CD8^+$ T cells ↗ functional NY-ESO-1 specific $CD4^+$ T cells, expansion of high-avidity $CD4^+$ T cells ↗ Th2 and Th9 cytokines	3 CR, 10 PD
		Montanide-ISA-51 VG	SC co-ad	≤ AE2, ↗ injection site reactions	↗, faster and broader IgG response (10/11) 9/11 functional $CD8^+$ T cells, 7/11 consistent & sustained Accelerated induction and ↗↗ functional NY-ESO-1 specific $CD4^+$ T cells, polyclonal, ↗ epitope spreading, expansion of high-avidity $CD4^+$ T cells ↗ Th1 cytokines, ↘ Th2 cytokines, no Th9 differentiation	2 CR, 9 PD ↗ TTP in NY-ESO-1^+ tumor patients

Table 3 (continued)

Reference	Indication	n	Phase	Antigen source (administration route)	Combined adjuvant	Poly-ICLC administration route	Safety	Immunologcal effects	Clinical effects
Kimura, 2013 (Kimura et al., 2013) (NCT00773097)	History of advanced adenomatous polyps	39	Feasi-bility	MUC1 peptide (SC)	n.a.	SC co-ad	Only AE1	MUC1-specific IgG in 17/39 Immune memory in 12/16 ↗ blood MDSC in non-responders; = Tregs	n.d.
Dhodapkar, 2014 (Dhodapkar et al., 2014) (NTC00948961)	Advanced malignancies	15/45*	I	CDX-1401 fusion protein (ICUT)	Resiquimod	n.a.	≤ AE2	↗ NY-ESO-1 specific IgG1	10/30 SD
					n.a.	SC concomitant and later	≤ AE2	↗↗ NY-ESO-1 specific IgG1	2/5 SD
					Resiquimod	SC concomitant and later	≤ AE2	↗ NY-ESO-1 specific IgG1	1/7 SD

Abbreviations: ¥, study ongoing; *, fracture designates the number of patients treated with poly-ICLC out of the total number of participants in the study; Ω, results of patients treated with a DC vaccine adjuvanted by either poly-ICLC or imiquimod; αDC1, α-type 1 polarized dendritic cells; ab, antibody; AE, adverse effect grade; boosters, booster injections; co-ad, combined administration of antigen source and adjuvant; CR, complete response; DC, dendritic cell; EphA2, ephrin type-A receptor 2; GAA, glioma-associated antigens; GM-CSF, granulocyte-macrophage colony-stimulating factor; gp100, glycoprotein 100; hCG-β, human choriogonadotrophin β; HGG,

high grade glioma; ICUT, intracutaneous; ID, intradermal; IL-13Rα2, interleukin-13 receptor subunit alpha-2; IM, intramuscular; IN, intranodal; IV, intravenous; LGG, low grade glioma; ManR, mannose receptor; MDSC, myeloid-derived suppressor cells; Mes, mesenchymal gene expression signature; MR, minor response; MUC1, mucin 1; n, number of participants; n.a., not applicable; n.d., not determined; NY-ESO-1, autoimmunogenic cancer/testis antigen 1; OLP, overlapping long peptides; OS, overall survival; PD, progressive disease; PN, proneural gene expression signature; Poly-ICLC, poly(I:C) stabilized with poly L-Lysine and carboxymethylcellulose; PR, partial response; SC, subcutaneous; SD, stable disease; Th1/2, helper T cell type 1/2; TIL, tumor-infiltrating lymphocytes; TLR, Toll-like receptor; Treg, regulatory T cell; TT, tetanus toxoid; TTP, time to progression; w/o, without; WHO, World Health Organization; WT1, Wilms' tumor 1; YKL-40, chitinase-3-like protein 1.

Supplementary table 1. Ongoing trials with poly-ICLC as cancer vaccine adjuvant on clinicaltrials.gov (15th June 2014)

Trial identifier	Indication	n^{estim}	Phase	Antigen source (administration route)	Combined adjuvant	Poly-ICLC administration route
Poly-ICLC as a single adjuvant						
NCT01677962	Locally advanced unresectable pancreatic adenocarcinoma	12	0	DC (IT)	n.a.	IT co-ad
NCT01734564	Solid tumors	25	II	Autologous DC (n.s.)	n.a.	n.s.
NCT01204684	HGG	60	II	Autologous tumor lysate-pulsed DC (ID)	n.a.	IM concomitant
NCT00766753	Recurrent HGG	30	I/II	Multiple GAA-pulsed αDC1 (IN)	n.a.	IM concomitant and later
NCT01130077	Pediatric glioma	60	Pilot	GAA peptides (n.s.)	n.a.	n.s.
NCT00874861	Adult recurrent LGG	10	0	GAA peptides (SC)	n.a.	IM concomitant and later
NCT00694551	Treated for prostate cancer	29	Pilot	PSMA and TARP peptide (n.s.)	n.a.	SC co-ad
NCT01920191	Newly diagnosed glioblastoma	16	I/II	IMA950 peptide (ID)	n.a.	IM
NCT01720836	Non-small cell lung cancer	30		MUC1 peptide (SC)	n.a.	SC co-ad
NCT00986609	Triple-negative breast cancer	37	0	MUC1 peptide (SC)	n.a.	IM concomitant
NCT02134925	Newly diagnosed advanced adenomatous polyps	120	II	MUC1 peptide (SC)	n.a.	SC co-ad
NCT01842139	AML in remission	36	I	$WT1_{126-134}$ peptide (SC)	n.a.	SC co-ad

Supplementary table 1 (continued)

Trial identifier	Indication	n^{estim}	Phase	Antigen source (administration route)	Combined adjuvant	Poly-ICLC administration route
NCT01718899	Smoldering multiple myeloma	22	I/IIa	PVX-410: 4 peptides (SC)	n.a.	IM concomitant
NCT01970358	Melanoma	20	I	NeoVax: personalized TAA peptides (n.s.)	n.a.	n.s.
NCT02149225	Newly diagnosed glioblastoma	20	I	APVAC1: 5-10 individually assembled glioma peptides (ID) APVAC2: 1-2 personalized mutanome peptides (ID)	n.a.	SC concomitant
NCT01834248	MDS or low blast count AML	15	I	CDX-1401 fusion protein (SC/ID)	n.a.	SC concomitant
NCT02129075	Melanoma	60	II	CDX-1401 fusion protein (SC/ID) CDX-301 (SC)	n.a.	SC concomitant with CDX-1401
NCT01976585	Low-grade B-cell lymphoma	30	I/II	CDX-301 (IT)	n.a.	IT concomitant
Poly-ICLC combined with other adjuvants						
NCT01437605	Resected MAGE-A3^{+} stage IV melanoma	44	II	MAGE-A3 autologous stem cell (n.s.)	AS15 (CpG ODN 7909, MPLA, QS-21)	n.s.

Supplementary table 1 (continued)

Trial identifier	Indication	n^{estim}	Phase	Antigen source (administration route)	Combined adjuvant	Poly-ICLC administration route
NCT02126579	Resected stage IIb-IV melanoma	58	I/II	LPV: 7 long peptides (ICUT)	Resiquimod Montanide-ISA-51 TT	ICUT concomitant
NCT01008527	Resected stage III or IV melanoma	22	I	NY-ESO-1 and gp100 peptide (SC)	CP 870,893 (CD40 agonist mAb) Montanide-ISA-51 VG	SC concomitant
NCT01079741	High-risk melanoma	33	I/II	NY-ESO-1 peptide (SC)	Montanide-ISA-51 VG	SC co-ad
NCT01810016	Unresectable or metastatic melanoma	27	I	NY-ESO-1 protein (SC) NY-ESO-1 OLP4 (SC)	Montanide-ISA-51 VG	SC co-ad
NCT00795457	Adult LGG	13	0	GAA/TT-peptides (SC)	Montanide-ISA-51	IM concomitant and later
NCT01585350	Melanoma	51	I	12 MELITAC 12.1 peptide (SC/ID/TD)	Montanide-ISA-51 TT	SC/ID/TD co-ad
NCT01846143	Melanoma	24	I	1 or 2 phosphopeptides (SC/ID)	Montanide-ISA-51 VG TT	SC/ID concomitant
NCT01532960	Early stage breast cancer (IB-IIIA)	24	Pilot	9 peptides: derived from HER2, CEA and CTA (IM/ID)	TT	IM/ID co-ad

Supplementary table 1 (continued)

Trial identifier	Indication	n^{estim}	Phase	Antigen source (administration route)	Combined adjuvant	Poly-ICLC administration route
NCT00374049	Recurrent and/or advanced prostate cancer	30	I	MUC1 peptide (SC)	GM-CSF TT	IM before and concomitant
NCT01245673	Advanced myeloma	28	II	MAGE-A3 peptide (n.s.)	GM-CSF	n.s., co-ad

Abbreviations**:** AML acute myeloid leukemia; APVAC, actively personalized vaccination; ASCI, autologous stem cell injection; ASCT, autologous stem cell transplant; CDX-1401, fusion protein of DEC-205 and NY-ESO-1; CEA, carcinoembryonic antigen; co-ad, combined administration of antigen source and adjuvans; concomitant, concomitant administration of antigen source and adjuvans; CTA, cancer/testis antigen; CTX, cyclophosphamide; DC, dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; HER2(+)/neu, human epidermal growth factor receptor 2 (positive); HGG, high grade glioma; spE7, fusion of heat shock protein Hsp65 from Bacillus Calmette-Guerin to E7 protein from human papilloma virus 16; ICUT, intracutaneous; ID, intradermal; IFN, interferon; IM, intramuscular; IT, intratumoral; IV, intravenous; LGG, low grade glioma; MAGE-A3(+), melanoma-associated antigen 3 (positive); MDS, myelodysplastic syndrome; MUC1, mucin 1; n^{estim}, estimated enrollment; n.a., not applicable; NY-ESO-1, autoimmunogenic cancer/testis antigen 1; OC-L, oxidized tumor cell lysate; OLP, overlapping long peptides; Poly-ICLC, polyinosinic:polycytidylic acid stabilized with poly L-Lysine and carboxymethyl cellulose; PSMA, prostate specific membrane antigen; PT, peritumoral; SC, subcutaneous; TARP, T-cell receptor γ alternate reading frame protein; TD, transdermal; TLR3, Toll-like receptor 3; TMZ, temozolomide; Top, topical; TT, tetanus toxoid; WT1, Wilms' tumor 1 protein.