Double-Stranded RNA Immunomodulators in Prostate Cancer

Andres M. Salazar, MD[a,*], Esteban Celis, MD, PhD[b]

KEYWORDS

• dsRNA • Prostate cancer • Immunotherapy • Poly-ICLC

KEY POINTS

- Relatively simple synthetic double-stranded RNAs can be powerful viral pathogen-associated molecular pattern (PAMP) mimics, inducing a broad panoply of antiviral and antitumor responses that act at multiple stages of the host defense.
- Their mechanisms of action and uses only now are beginning to be understood and appreciated in the clinic, alone, in combination with other therapeutics, or as relatively novel PAMP adjuvants providing the critical danger signal that has been missing from most cancer and other modern vaccines.
- As with other immunomodulators, dose, timing, route of administration combinations, and other clinical variables can have a critical impact on immunogenicity, particularly in the management of prostate cancer and other tumors.

BACKGROUND

The natural history of prostate cancers is described elsewhere in this volume. In spite of advances in surgery, radiotherapy, and hormonal and targeted therapies, management of all stages of prostate cancer still holds many challenges. The most recent approach is the emerging variety of active immunotherapies discussed in this article. These are based on the power of the immune system and a long-standing and still elusive question: if mutated tumor proteins (neoantigens) are indeed foreign, what leads to the failure of the immune surveillance that allows cancers to develop and persist? In other words, what are the myriad evasive mechanisms used by cancers to survive, and what host counter-defenses have evolved with them can be used to advantage? Similarly, why has there been a relative failure of vaccines that utilize tumor-associated antigens (eg, prostate-specific membrane antigen) and neoantigens?

Some of these answers may lie in how the host responds to viral infections, notably a re-recognition of the importance of danger signals or pathogen-associated molecular patterns (PAMPs) that activate innate and adaptive immune responses and can serve either as vaccine adjuvants or as monotherapies. Another important breakthrough is the recognition of tumor evasion through up-regulation of immune checkpoints in the tumor microenvironment and the advent of checkpoint blockers, especially anti–PD-1 and anti–PD-L1 antibodies, discussed later. This article briefly introduces a similarly promising approach to active immunotherapy that uses synthetic dsRNA viral mimics, notably polyinosinic-polycytidylic acid (poly-IC) derivatives, such as rintatolimod and poly-ICLC (Hiltonol, Oncovir, Inc, Washington, DC), with an emphasis on the latter.

Poly-IC is a relatively simple, synthetic, double-stranded RNA (dsRNA), first developed more than 50 years ago by Merck, that has been long

[a] Oncovir, Inc, 3203 Cleveland Avenue Northwest, Washington, DC 20008, USA; [b] Department of Medicine, Medical College of Georgia, Oncovir, Inc, 1410 Laney Walker Boulevard, CN4121, Augusta, GA 30912, USA
* Corresponding author.
E-mail address: asalazar@oncovir.com

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recognized as an interferon type I (IFN-I) inducer, immunomodulator, and antiviral and antitumor agent as well as a potent vaccine adjuvant danger signal in mice. dsRNAs typically are not found in the body but are transient products of viral replication that alert and help the immune system distinguish between harmless foreign proteins, such as food and more genuine threats. Poly-IC has been characterized as a PAMP mimic that binds to toll-like receptor (TLR) 3 and the retinoic acid inducible gene I–like products MDA5 and RIG-I, all of which serve as pathogen recognition receptors. This topic still is at the base of an extensive literature that has expanded knowledge of the immune system but that is beyond the scope of this brief review.

It was soon recognized, however, that the multiple effects of plain poly-IC in mice could not be replicated in some species, neither in humans nor in nonhuman primates. It then was recognized that this largely was related to rapid breakdown of dsRNA by abundant serum and tissue ribonucleases (RNases). Approximately 45 years ago, Levy and colleagues, then at the National Institute of Allergy and Infectious Diseases, stabilized poly-IC with poly-L-lysine and carboxymethylcellulose, showing that the resulting compound, poly-ICLC, also known as Hiltonol, was RNAse resistant and a very powerful IFN-I inducer in monkeys, chimpanzees, and humans.[1,2]

Poly-ICLC sometimes was referred to as a poor man's interferon and initially was pushed to a maximal tolerated dose of approximately 300 μg/kg in early cancer trials, in which it induced large amounts of IFN-I (approximately 4400 IU/mL.).[3] This resulted in significant yet transient toxicity and mixed results, and the compound subsequently was dropped by industry in favor of the recombinant IFN-I.

One response to the interferon-related toxicity seen with the initial high-dose trials of poly-ICLC was development of a mismatched poly-IC, poly I:poly C12U (Ampligen), that was broken down more rapidly by the body and apparently activates only TLR3 and not MDA5 and RIG-I. This was a less toxic but weaker IFN-I inducer and it still is in widespread experimental clinical trials, including for prostate cancer, albeit at much higher doses intravenously (IV) in combination with aspirin and IFN-I. Further discussion of these also is beyond the scope of this brief review.[4]

It later was found that doses of poly-ICLC, as low as 10 μg/kg to 20 μg/kg, when given intramuscularly (IM), were very well tolerated and generated much broader and seemingly more effective immunomodulation in humans.[5,6] Subsequent clinical cancer trials using poly-ICLC IM, subcutaneously (SC), intratumorally (IT), or nasally at those nontoxic doses, alone or in combination with vaccine antigens, and other immunomodulators have further confirmed its clinical safety.

At the same time, some of poly-ICLC's basic mechanisms of action have been clarified more fully, including its induction/activation of a natural mix of interferons, cytokines, chemokines, costimulators, natural killer (NK) cells, dendritic cells, and CD4 and CD8 T cells. As a PAMP, poly-ICLC signals through various dsRNA-dependent host-defense systems, such as the 2′5′OAS, PKR, TLR3, RIG-I, and MDA5, some of which are discussed later. It must be emphasized, however, that at a clinical level, dose, route, timing, combinations, and other details of administration can be essential to optimal immunomodulation, especially in the presence of cancer or viral infections that generate their own immune evasions.[7]

POLY-ICLC DUAL SIGNALING AND MULTIPLE ACTIONS AT VARIOUS STEPS OF THE HOST DEFENSE

Building on earlier clinical and dose finding work, in a systems biology study, Ralph Steinman's group at Rockefeller University showed that a single dose of 1.6-mg poly-ICLC, given either SC or intranasally in human volunteers, consistently activates several hundred genes, representing some 10 canonical innate immune pathways.[8] The transcriptome pattern generated closely mimics that of a highly effective attenuated live-virus yellow fever vaccine (**Fig. 1**), further confirming poly-ICLC as a reliable and authentic therapeutic viral mimic in humans.[8] The clinical implications of these findings are considerable and have yet to be fully exploited, but in the present context it is useful to consider poly-ICLC parallel activation of 2 signaling pathways, TLR3 and MDA5.[9,10]

It now appears that partly because of its dual TLR3 and MDA5 activation, poly-ICLC acts at various stages of immunity. Perhaps its best studied action as a vaccine adjuvant is its priming of an immune response through its activation of myeloid dendritic cells and various costimulators. This PAMP-adjuvant priming effect also can be induced by several TLR ligands, including CpG, and various other dsRNAs, such as plain poly-IC and Ampligen, as well as by poly-ICLC via TLR3, inducing IFN-I and interleukin (IL)-12.[11] Thus, several poly-ICLC adjuvanted vaccine trials currently are ongoing and are discussed later.

Beyond the initial immune prime, however, poly-ICLC's relatively unique activation of the MDA5 and IFN-I pathways then is needed for CD8 T cell expansion through IL-15 and specific CD8 T-cell

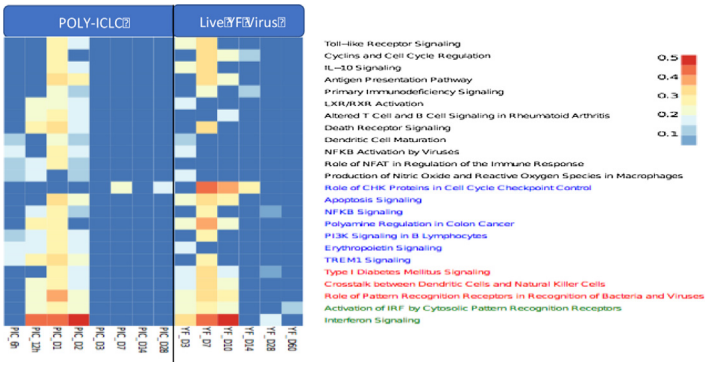

Fig. 1. Poly-ICLC, a reliable and authentic viral mimic in humans. Transcriptome induced by single SC dose of poly-ICLC versus yellow fever vaccine in healthy volunteers. Ratio of regulated to pathway genes. (*Adapted From* Caskey M., Lefebvre F., Filali-Mouhimet A., et al. Synthetic double stranded RNA reliably induces innate immunity similar to a live viral vaccine in humans. J Exp Med, 2011;208(12):2357-66. https://doi.org/10.1084/jem.20111171.)

tumor targeting and infiltration (later) as well as for a previously unknown anti-inflammatory effect in ischemic stroke and in certain viral infections, such as severe acute respiratory syndrome and possibly coronavirus disease 2019.[11–14] The CD8 T-cell expansion is not seen or not nearly as pronounced with plain poly-IC, other dsRNAs, or other TLR ligands. Recently, the authors' group confirmed that poly-ICLC's action is mediated in part not through the traditional endosomal TLR3 receptor but by preferential activation of the cytoplasmic dsRNA-dependent MDA5 helicase.[15] MDA5 also is particularly sensitive to long-chain dsRNA, as in poly-ICLC. As importantly, this preferential MDA5 activation also is made possible by the serendipitous use of poly-lysine, a known transfection agent, as the stabilizer of the poly-IC molecule. The presence of poly-lysine in poly-ICLC results in lysis of the endosome through a proton-sponge effect, releasing poly-ICLC into the cytoplasm, where it can activate MDA5. The expansion of CD8 T cells with or without vaccine is facilitated further by systemic (IM or IV) as opposed to SC administration, possibly because those routes recruit higher numbers of CD8 T-cell precursors throughout the body.[9,11,16]

In addition, some years ago, Okada and colleagues demonstrated a poly-ICLC–induced IFN-I and chemokine–dependent targeting and infiltration of tumors by CD8 cytotoxic T cells.[17] Most recently, the authors confirmed and considerably extended these findings. In an MDA5/IFN-I–dependent manner, paired doses of IM but not IT poly-ICLC markedly enhanced infiltration of antigen-specific CD8 T cells into tumor through the blood-tumor barrier. This is mediated through a direct effect of systemic poly-ICLC on tumor vascular endothelium and an increase in IFN-I and vascular cell adhesion molecule 1.[18] But, in addition, the authors found a concurrent poly-ICLC–induced marked decrease of myeloid-derived suppressor cells (MDSCs) in the tumor. The mechanism behind the simultaneous decrease in MDSCs is not yet clear, but it may involve a reprogramming of the microenvironment in prostate cancer or a poly-ICLC–induced differentiation of MDSCs into nonsuppressive matured myeloid cells.[19,20]

The immediate clinical implications of these findings are at least 2-fold. First, regardless of whether sensitized CD8 T cells are generated by vaccination, by autovaccination (discussed later), or by adoptive cell therapies, including chimeric antigen receptor T-cell therapies, paired IM maintenance treatments with IM or IV poly-ICLC facilitate their infiltration into the tumor parenchyma. It is not clear for how long such treatments might be necessary to maintain this effect, but vaccine studies suggest that it can be months to years.[21] Another potential benefit of such continued maintenance might be kindling of epitope spread with a more durable response against mutating tumor neoantigens released from dying tumor cells, especially in combination with PD-1 blockade.[21]

A second implication of the authors' recent report is that the decrease in MDSCs suggests that preconditioning for a couple of weeks with IM poly-ICLC may enhance generation of vaccine immunity in some cases. An older study demonstrated that such preconditioning facilitates response to a MUC1 vaccine in 9 out of 14 immunosuppressed patients with advanced prostate cancer.[22]

POLY-ICLC CLINICAL MONOTHERAPY

Since the original clinical trials at the maximal tolerated dose in advanced cancer patients, a variety of trials have explored the use of lower-dose poly-ICLC in various cancers, especially gliomas, as well as in human immunodeficiency (HIV)/acquired immunodeficiency syndrome and multiple sclerosis. Initial moderate dose trials used IM or IV poly-ICLC in more than 60 patients with very

advanced renal cell carcinoma, lymphomas, and other solid cancers, at doses ranging from 100 μg/kg to 250 μg/kg. These doses produced some toxicity but generally were well tolerated. Stable disease was reported in 6/29 renal carcinomas but there were no partial responses or complete responses, and this indication has not been pursued further. It appears that even these moderate doses may have been too high.[23]

Even lower-dose, IM poly-ICLC alone also has been used in malignant gliomas as well as in low-grade gliomas. An early dose-ranging study treated approximately 38 glioblastoma, anaplastic glioma, and recurrent malignant glioma patients, with IM doses ranging from 10 μg/kg to 50 μg/kg, once, twice, or thrice a week for as long as 52 months. Treatment was well tolerated. Disease control rate was 100% in a study for anaplastic glioma patients, with a median progression-free survival of 54 months and a 5-year survival of 90%. Median survival for glioblastoma patients was 19 months.[24] Three National Cancer Institute consortia trials confirmed these findings in more than 180 patients.[25,26] There was a tail of approximately 25% of patients surviving longer than 36 months, not unlike the proportion of long-term survivors on other immunotherapies. In a pediatric glioma study, patients with lower-grade gliomas responded better than those with high-grade tumors.[27] In a recent confirmatory study, lower-grade glioma patients with inoperable rapidly progressing recurrent gliomas were treated with poly-ICLC, at 20 μg/kg IM, twice weekly, for 2 years. Patients tolerated treatment well. Preliminary analysis suggests that progression-free survival was approximately 70%, 52%, and 48% at 6 months, 24 months, and 48 months, respectively. Many have remained stable months after study end at 2 years (Aguilera 2020, unpublished data) A follow-up trial in neurofibromatosis type 1-associated pediatric low-grade gliomas is ongoing.

POLY-ICLC AS A VACCINE ADJUVANT—CLINICAL TRIALS

As discussed previously, poly-ICLC is an authentic and reliable viral mimic in humans and it provides the dsRNA PAMP danger signal normally furnished by viral replication but that has been missing from most modern vaccines for cancer, HIV, malaria, and other diseases. In other words, when properly combined with antigen, poly-ICLC can generate a live-virus vaccine equivalent with a comprehensive immune response that includes activation of myeloid dendritic cells, other antigen-presenting cells, and NK cells and generation of a polyfunctional type 1 helper T-cell–polarized CD4 T-cell and CD8 T-cell response, which, via the induction of specific chemokines, can home to tumor or pathogen.[4,28,29] Some of these basic mechanisms are described previously.

Poly-ICLC thus is emerging as an immunogenic core for multiple cancer and HIV vaccines. One example is the autovaccination strategy, described later. But, in addition, approximately several dozen clinical trials have been using poly-ICLC in combination with peptide, protein, dendritic cell, and dendritic cell–targeted vaccines. These have demonstrated enhanced specific immune responses as well as notable clinical tumor responses.[30–38]

In Situ Therapeutic Autovaccination with Sequential Intratumoral and Intramuscular Poly-ICLC

Most cancer vaccines use 1 or more exogenously administered tumor-associated antigens or neoantigens, but another host-targeted strategy attempts to immunize against the patient's own tumor antigens in situ. Sequential IT and IM poly-ICLC, CpG, oncolytic virus, or other injections mimicking a viral infection within the tumor microenvironment also can induce an effective, in situ, personalized systemic therapeutic autovaccination against a patient's individual tumor antigens.[39–42] In cases of poly-ICLC, this strategy may involve several steps. First is activation of NK cells, various cytokines, TLR3, PKR, and other proapoptotic mechanisms, resulting in early tumor killing and antigen release.[43,44] Second, in the same local context, is recruitment and activation of myeloid dendritic cells and macrophages at the tumor site, where they acquire tumor antigens that are being released, present them to CD4 T helper cells, and cross-present them to CD8 T cells in the tumor or in regional and systemic lymph nodes to generate antigen-specific T cells. The repeated administration of the PAMP danger signal IT in the context of the patient's own tumor antigens and in a way that mimics a natural viral infection also has been used successfully with IT CpG and may be critical to this step.[39] Timed-release formulations of poly-ICLC could mimic a viral infection even better, especially for deep tumors where repeated IT administration is difficult. The third step is remote targeting and maintenance of antigen-specific CD8 T cells via various poly-ICLC–induced chemokines, costimulatory factors, and the T-cell infiltration mechanisms discussed previously.[17,18] Three illustrative cases are presented.

The first was a young man in hospice with an exceptionally advanced and heavily pretreated facial and oral embryonal rhabdomyosarcoma with extension to brain. He was treated with repeat doses of IT and IM poly-ICLC and initially showed tumor inflammation or pseudoprogression consistent with immunotherapy, which was treated transiently with low-dose dexamethasone. Such inflammation with a gradual tumor response is typical of many immunotherapies. This was followed by gradual marked tumor necrosis and regression, with extended functional survival (6 months). At time of death due to cerebral complications, he had no evidence of peripheral tumor.[40]

The second was a 60-year-old woman with recurrent diffuse cutaneous T-cell lymphoma who responded to sequential IT and IM poly-ICLC targeting abdominal cutaneous lesions over 6 months. Necrosis and inflammation of previously undetected remote micrometastases were seen at approximately weeks 2 to 3. The lesions resolved spontaneously, confirming pseudoprogression and suggesting an effective systemic immune response (**Fig. 2**). She was in complete response at the end of treatment (week 26), recurred after conclusion of the protocol, but again resolved on pembrolizumab. She remains in complete response 5 years post-treatment.

The third was a 54-year-old man with recurrent head and neck squamous cell cancer who showed clinical benefit after 2 cycles of sequential IT and IM poly-ICLC. Immunohistochemistry showed the tumor turning from immunologically cold to hot, with increased PD-L1 expression (20×), together with increases of CD4 T cells (60×), CD8 T cells (10×), NK cells, and CD89 and CD68 macrophages (**Fig. 3**).[9,42]

In summary, autovaccination using sequential IT plus IM poly-ICLC has been shown safe and well tolerated. Indications of efficacy in very advanced, heavily pretreated patients suggest that additional studies are warranted using this regimen alone or in combination with PD-1 blockade inhibitors or other immunomodulators, including in more selected patients with less advanced disease.

Several additional clinical trials have been pursuing this strategy in hepatoma, solid cancers, and lymphoma with or without additional anti–PD-1 antibodies.[45,46]

POLY-ICLC IN PROSTATE CANCER, CLINICAL TRIALS

Sequential Intratumoral and Intramuscular Poly-ICLC in Prostate Cancer—2 Clinical Trials

Based on the considerations, discussed previously, a phase I dose-ranging safety trial of IT plus IM neoadjuvant poly-ICLC in patients with high-risk locally contained prostate cancer scheduled for radical prostatectomy (International Society of Urological Pathology [ISUP] Gleason groups 3, 4, and 5) currently is ongoing at the Department of Urology at the Icahn School of Medicine at Mount Sinai. Patients receive 1 or 2 IT injections followed by twice-weekly IM of poly-ICLC for 4 weeks, followed by radical prostatectomy. To date, 7/7 treated patients have tolerated treatment well, reaching a dose of 1.0 mg/IT injection in the third cohort. Patients did not experience treatment-associated adverse events. Additional patients will be treated at the appropriate escalating doses (NCT03262103).

Based on the preliminary safety findings, a second, single-blind, randomized placebo-controlled phase II trial with poly-ICLC is being investigated for prostate cancer patients in ISUP Gleason groups 1 and 2 who are followed on active surveillance. Patients will receive sequential IT and then IM poly-ICLC over 48 weeks. The primary endpoint is reduction or stabilization of the Gleason score at 1 year.

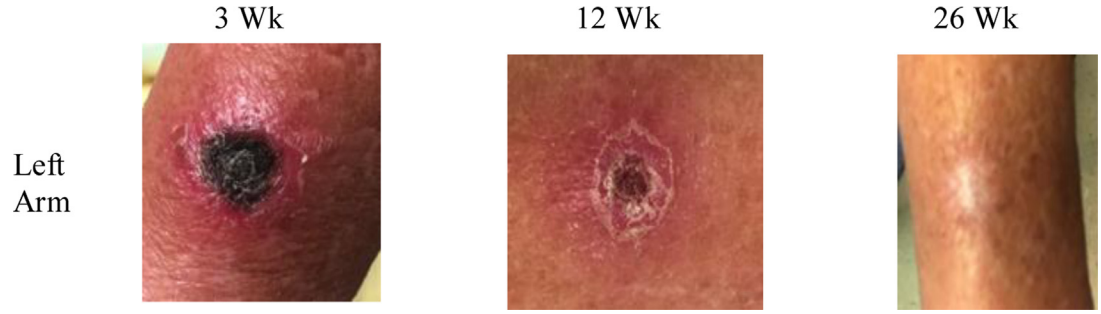

Fig. 2. IT poly-ICLC in cutaneous T-cell lymphoma. Inflammation and spontaneous regression of previously undetected remote micrometastases (see text). (*From* Sultan H., Salazar AM., Celis E. Poly-ICLC, a multi-functional immune modulator for treating cancer. Seminars in Immunology 2020; in press.)

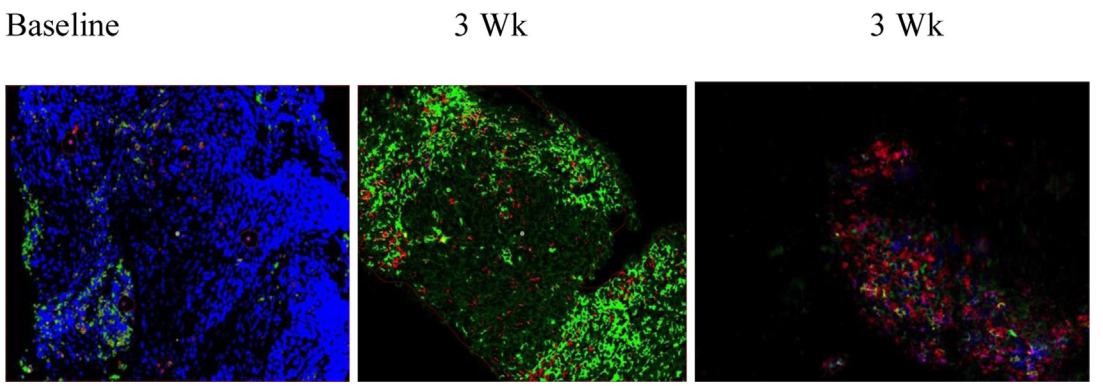

Fig. 3. Immunohistochemistry of head and neck squamous cell cancer pre-IT and post-IT. Poly-ICLC: PD-L1, green; CD8, red; and CD4, green (see text). (*From* Sultan H., Salazar AM., Celis E. Poly-ICLC, a multi-functional immune modulator for treating cancer. Seminars in Immunology 2020; in press; and Kyi C, Vladimir Roudko, Rachel Sabado, et al. Therapeutic immune modulation against solid cancers with intratumoral poly-ICLC: a pilot trial. Clin Cancer Res 2018;24(20):4937–48.)

Combination with Costimulators: anti–PD-1 Antibody and FLT3

All in all, these results suggest that poly-ICLC alone may be most helpful in relatively early mid-grade to low-grade tumors. Combination with PD-1 blockade inhibitors, however, also has shown a potent synergy in murine models of melanoma, Lewis lung cancer, and M38 colon cancer.[47] These currently are under evaluation in ongoing clinical trials that have demonstrated safety to date.[45] A clinical trial combining FLT3, poly-ICLC, and anti–PD-1 antibody (nivolumab) for patients with metastatic prostate cancer currently is under way (NCT03835533).

SUMMARY

Relatively simple synthetic dsRNAs can be powerful viral (PAMP) mimics, inducing multiple distinct elements of antiviral and antitumor responses functioning at multiple stages of the host defense. Their mechanisms of action only now are coming into focus, as are their uses in the clinic alone, in combination with other costimulators, or as relatively novel vaccine PAMP adjuvants. Early clinical trials are beginning to demonstrate their safety and potential utility in generating effective immune responses against prostate cancer.

DECLARATION OF COMPETING INTEREST

A.M. Salazar is President and CEO of Oncovir, Inc., which is developing poly-ICLC (Hiltonol) for clinics. E. Celis is a paid consultant for Oncovir, Inc. This work was partly funded by a grant from the National Cancer Institute, Small business innovative research (SBIR) program.

REFERENCES

1. Levy H, Baer G, Baron S, et al. A Modified polyriboinosinic-polyribocytidylic acid complex that induces interferon in primates. J Infect Dis 1975; 132:434–9.
2. Levy H, Riley FL, Lvovsky E, et al. Interferon induction in primates by stabilized polyriiboinosinic acid-polyribocytidylic acid: effect of component size. Infect Immun 1981;34(2):416–21.
3. Levine A, Sivulich M, Wiernik PH, et al. Initial clinical trial in cancer patients of polyriboinosinic-polyribocytidylic acid stabilized with poly-L-lysine, in carboxymethylcellulose [poly(ICLC)], a highly effective interferon inducer. Cancer Res 1979;39:1645–50.
4. Martins K, Bavari S, Salazar A. Vaccine adjuvant uses of poly-IC and derivatives. Expert Rev Vaccines 2014;14(3):447–59.
5. Maluish A, Reid JW, Crisp EA, et al. Immunomodulatory effect of Poly-ICLC in cancer patients. J Biol Response Mod 1985;4:656–63.
6. Talmadge J, Adams J, Phillips H, et al. Immunotherapeutic potential in murine tumor models of polyinosinic-polycytidylic acid and poly-L-lysine solubilized by carboxymethylcellulose. Cancer Res 1985;45:1066–72.
7. Garcia-Sastre A. Ten strategies of interferon evasion by viruses. Cell Host Microbe 2017;22(2): 176–84.
8. Caskey M, François L, Abdelali F-M, et al. Synthetic double stranded RNA reliably induces innate immunity similar to a live viral vaccine in humans. J Exp Med 2011;208(12):208.
9. Sultan H, Salazar AM, Celis E. Poly-ICLC, a multi-functional immune modulator for treating cancer. Semin Immunol 2020. https://doi.org/10.1016/j.smim.2020.101414.
10. Palchetti S, Donatella S, Paola De C, et al. Transfected poly(I:) activates different dsRNA receptors,

leading to apoptosis or immunoadjuvant response in androgen-independent prostate cancer cells. J Biol Chem 2015;290(9):5470–83.
11. Sultan H, Valentyna IF, Diane A, et al. Designing therapeutic cancer vaccines by mimicking viral infections. Cancer Immunol Immunother 2016;66(2):203–13.
12. Packard A, Jason CH, Frances RB, et al. Poly-IC preconditioning protects against cerebral and renal ischemia-reperfusion injury. J Cereb Blood Flow Metab 2011;32(2):242–7.
13. Gesuete R, Sara NC, Frances RB, et al. Cytosolic receptor melanoma differentiation–associated protein 5 mediates preconditioning-induced neuroprotection against cerebral ischemic injury. Stroke 2016; 47:262–6.
14. Kumaki Y, Andres MS, Miles KW, et al. Prophylactic and therapeutic intranasal administration with an immunomodulator, Hiltonol((R)) (Poly IC:LC), in a lethal SARS-CoV-infected BALB/c mouse model. Antiviral Res 2017;139:1–12.
15. Sultan H, Juan W, Takumi K, et al. Role of MDA5 and interferon-1 in dendritic cells for T cell expansion by anti-tumor peptide vaccines in mice. Cancer Immunol Immunother 2018;67:1091–103.
16. Sultan H, Takumi K, Toshihiro N, et al. The route of administration dictates the immunogenicity of peptide-based camcer vaccines in mice. Cancer Immunol Immunother 2019;68(3):455–66.
17. Zhu X, Fallert-Junecko BA, Mitsugu F, et al. Poly-ICLC promotes the infltration of effector T cells into intracranial gliomas via induction of CXCL10 in IFN-a and IFN-g dependent manners. Cancer Immunol Immunother 2010;59:1401–9.
18. Sultan H, Juan W, Valentyna IF, et al. Poly-IC enhances the effectiveness of cancer immunotherapy by promoting T cell tumor infiltration. J Immunother Cancer 2020;8:e001224.
19. Metzger P, Sabrina VK, Michael K, et al. Immunostimulatory RNA leads to functional reprogramming of myeloid derived suppressor cells in pancreatic cancer. J Immunother Cancer 2019;7:288.
20. Muthuswamy R, John MC, Kathryn D, et al. Functional reprogramming of human prostate cancer to promote local attraction of effector CD8 T Cells. Prostate 2016;76(12):1095–105.
21. Memarnejadian A, Courtney EM, Christopher RS, et al. PD-1 Blockade promotes epitope spreading in anticancer CD8 T cell responses by preventing fratricidal death of subdominant clones to relieve immunodomination. J Immunol 2017;199(9):3348–59.
22. Patel AK, Appleman LJ, Gooding WE, et al. A dose-finding study of a MUC-1 vaccine in conjunction with poly-ICLC (polyinosinic-polycytidylic acid stabilized with polylysine and carboxy methylcellulose) in immunosuppressed (IS) patients (pts) with advanced prostate cancer (PCa). J. Clin Oncol, Volume 29, issue15_suppl (May 20, 2011) Abstract 2564.
23. Giantonio B, Hochster H, Blum R, et al. Toxicity and response evaluation of the interferon inducer poly-ICLC adminstered at low dose in advanced renal carcinoma and relapsed or refractory lymphoma. a report of two clinical trials of the Eastern Coop Oncology Group. Invest New Drugs 2001;19: 89–92.
24. Salazar AM, Levy HB, Ondra S, et al. Long-term IM Poly-ICLC treatment of malignant glioma: an open pilot study. Neurosurgery 1996;38(6):1096–104.
25. Rosenfeld M, Marc CC, Stuart AG, et al. A multi-institution phase II study of poly- ICLC and radiotherapy with concurrent and adjuvant temozolomide in adults with newly diagnosed glioblastoma†. Neuro Oncol 2010;12(10):1071–7.
26. Butowski N, Susan MC, Larry J, et al. A phase II clinical trial of poly-ICLC with radiation for adult patients with newly diagnosed supratentorial glioblastoma: a North American brain tumor consortium (NABTC01-05). J Neurooncol 2008;91(2):175–82.
27. Hartman L, John RC, Milan TM, et al. Pediatric phase II trials of Poly-ICLC in the management of newly diagnosed and recurrent brain tumors. J Pediatr Hematol Oncol 2014;36(6):451–7.
28. Black P, Hartmann D, Pennington R, et al. Effect of tumor burden and route of administration on the immunotherapeutic properties of polyinosinic-polycytidylic acid stabilitzed with poly-lysine in carboxymethylcellulose [poly(IC)-LC]. Int J Immunopharmacol 1992;14:1341–53.
29. Flynn B, Kathrin K, Ulrike W-R, et al. Immunization with HIV Gag targeted to dencritic cells followed by recombinant NY vaccinia virus induces robust Tcell immunity in non-human primates. Proc Natl Acad Sci U S A 2011;108(17):7131–6.
30. Okada H, Pawel K, Ryo U, et al. Induction of CD8 T-Cell responses against novel glioma–associated antigen peptides and clinical activity by vaccinations with a-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. J Clin Oncol 2010;28: 330–6.
31. Okada H, Kalinski P, Mintz AH, et al. Phase I/II vaccine study targeting novel HLA-A2-restricted CTL epitopes in combination with poly-ICLC in patients with recurrent malignant glioma., in ASCO Annual Meeting. Chicago: ASCO; 2011.
32. Okada H, Butterfield LH, Sakaki M, et al. A bi-institutional pilot study of peptide-based vaccines in combination with poly ICLC in patients with WHO grade 2 low-grade glioma, in Society Immunotherapy of Cancer. J. Clin Oncol Volume 29, issue 15 suppl. Abstract 2506. 2012: Bethesda.
33. Ott PA, Zhuting H, Derin BK, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature 2017;547(7662):217–21.

34. Rapoport A, Nicole AA, Edward AS, et al. Combination immunotherapy after ASCT for multiple myeloma using MAGE-A3/Poly-ICLC immunizations followed by adoptive transfer of vccine-primed and costimulated autologous T cells. Clin Cancer Res 2014;20(5):1355–65.
35. Kimura T, John RM, Lynda AD, et al. MUC1 vaccine for individuals with advanced adenoma of the colon: a cancer immunoprevention feasibility study. Cancer Prev Res (Phila) 2012;6(1):18–26.
36. Bhardwaj N, et al. Flt3 ligand significantly augments immune responses to a dendritic cell targeting anti-DEC-205 NY Eso1 vaccine through expansion of dendritic cell subsets. Nature Cancer, 2020. In Press.
37. Migliorini D, Valérie D, Mathilde A, et al. Phase I/II trial testing safety and immunogenicity of the multipeptide IMA950/poly-ICLC vaccine in newly diagnosed adult malignant astrocytoma patients. Neuro Oncol 2019;21(7):923–33.
38. Hilf N, Sabrina K-C, Katrin F, et al. Actively personalized vaccination trial for newly diagnosed glioblastoma. Nature 2018;565(7738):240–5.
39. Houot R, Levy R. T-cell modulation combined with intratumoral CpG cures lymphoma in a mouse model without the need for chemotherapy. Blood 2009; 113:3546–52.
40. Salazar A, Rodrigo BE, Alexander M, et al. Therapeutic in situ autovaccination agains solid cancer siwth intratumoral poly-ICLC: Case report, Hypothesis, and Clinical Trial. Cancer Immunol Res 2014; 2(8):720–4.
41. Marabelle A, Holbrook K, Christophe C, et al. Intratumoral immunization: a new paradigm for cancer therapy. Clin Cancer Res 2014;20(7): 1747–56.
42. Kyi C, Vladimir Roudko, Rachel Sabado, et al. Therapeutic immune modulation against solid cancers with intratumoral poly-ICLC: a pilot trial. Clin Cancer Res 2018;24(20):4937–48.
43. Chew V, Charlene T, Caleb H, et al. TLR 3 expressing tumor parenchyma and infiltrating natural killer cells in hepatocellular carcinoma patients. J Natl Cancer Inst 2012;104:1796–807.
44. Ho V, Tong SL, Justin L, et al. TLR3 agonist and Sorafenib combinatorial therapy promotes immune activation and controls hepatocellular carcinoma progression. Oncotarget 2015;6(29):27252–66.
45. Hammerich L, Thomas UM, Ranjan U, et al. Systemic clinical tumor regressions and potentiation of PD1 blockade with in situ vaccination. Nat Med 2019;25:814–24.
46. de la Torre AN, Contractor S, Castaneda I, et al. A Phase I trial using local regional treatment, nonlethal irradiation, intratumoral and system polyinosinipolycytidylic acid polylysine carboxymethilcellulose to treat liver cancer: in searh of the abscopal effect. J Hepatocellular Carcinoma 2017;4:111–21. https://doi.org/10.2147/JHC.S136652.
47. Nagato T, Young-Ran L, Yasuaki H, et al. Combinatorial Immunotherapy of Poly-IC and blockade of PD-L1 induce effective CD8 T-cell responses against established tumors. Clin Cancer Res 2014;20(5): 1223–34.