Therapeutic *In Situ* Autovaccination against Solid Cancers with Intratumoral Poly-ICLC: Case Report, Hypothesis, and Clinical Trial

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**Abstract**

Pathogen-associated molecular patterns (PAMP) are stand-alone innate and adaptive immunomodulators and critical vaccine components. We present a strategy of sequential intratumoral (i.t.) and intramuscular (i.m.) injections of the stabilized dsRNA viral mimic and PAMP, polyinosinic–polycytidylic acid-polylysine-carboxymethylcellulose (poly-ICLC, Hiltonol; Oncovir). We report the first treated patient, a young man with an exceptionally advanced facial embryonal rhabdomyosarcoma with extension to the brain. After treatment, the patient showed tumor inflammation consistent with immunotherapy, followed by gradual, marked tumor regression, with extended survival. Sequential i.t. and i.m. poly-ICLC injections mimicking a viral infection can induce an effective, *in situ*, personalized systemic therapeutic "autovaccination" against tumor antigens of a patient. We postulate a three-step immunomodulatory process: (i) innate-immune local tumor killing induced by i.t. poly-ICLC; (ii) activation of dendritic cells with Th1 cell– and CTL–weighted priming against the released tumor antigens; and (iii) i.m. poly-ICLC maintenance of the systemic antitumor immune response via chemokine induction, facilitation of CTL killing through the induction of costimulators such as OX40, inflammasome activation, and increase in the T-effector/Treg ratio. These results support the use of certain simple and inexpensive i.t. PAMPs to favorably stimulate effective immunity against solid cancers. A phase II clinical trial testing the hypothesis presented has begun accrual (clinicaltrials.gov, NCT01984892).

**Introduction**

Pathogen-associated molecular patterns (PAMP) are stand-alone innate and adaptive immunomodulators or "danger signals," and essential vaccine components. Polyinosinic–polycytidylic acid-polysyxine-carboxymethylcellulose (poly-ICLC, Hiltonol; Oncovir) is a stabilized double-stranded RNA (dsRNA), which is a viral mimic, a ligand to Toll-like receptor 3 (TLR3), and a PAMP. We present a strategy of sequential intratumoral (i.t.) and intramuscular (i.m.) injections of Hiltonol that can induce a personalized therapeutic "autovaccination" against a patient's individual tumor antigens, as processed *in situ*. We report the first case so treated, a patient with very advanced embryonal rhabdomyosarcoma, who is in hospice. The especially encouraging results support the use of certain IT PAMPs to favorably stimulate effective immunity against some advanced cancers. A solid-cancer clinical trial testing this hypothesis has been initiated.

**Materials and Methods**

In the advanced, compassionate-use case presented, Hiltonol (1 mg) was given i.t. twice weekly for 4 weeks followed by a 2-week rest period. In the subsequent two cycles, some injections had to be skipped or were given i.m.

In the ongoing clinical trial, 1 mg of Hiltonol is given i.t. three times a week for 2 weeks, followed by i.m. Hiltonol boosters twice weekly for 6 weeks. A second cycle is followed by a 6-week no-treatment period with evaluation of response at week 26.

**Case Report and Results**

The patient was an 18-year-old man with an exceptionally large malignant embryonal rhabdomyosarcoma, who failed eight different regimens of chemotherapy, including pazopanib and imetelstat, as well as radiotherapy and proton-beam therapy, and was on hospice. In the months before his volunteering for i.t. Hiltonol treatment approved under compassionate-use exemption, his tumor grew exponentially from his left cheek to fill and grow out of his mouth, expand to his left sinuses, nasal cavity, nasopharynx, left orbit, temporalis muscle, and into the brain. It involved most of his temporal lobe with extension to the frontal lobe, obliteration of the left lateral ventricle, and a marked mass effect with compromise of the left temporal lobe.
cerebral peduncle (Figs. 1 and 2). He required a tracheostomy and gastric tube feeding. The mouth and cheek tumor became necrotic by week 2 of the first treatment cycle (Fig. 2B). Left internal maxillary artery embolization was performed to control bleeding on week 3. The brain tumor also appeared on MRI to be necrotic, and at week 4 he was started on bevacizumab every 3 weeks to help control brain swelling. Formal immunomonitoring was not possible, but the patient developed marked local tumor inflammation after the first i.t. injection of his second cycle in week 7, prompting a brief course of dexamethasone. Local facial tumor inflammation was also observed after each subsequent injection of i.m. Hiltonol in the thigh, suggesting the induction of a systemic immune response (Fig. 2C). By week 7, the tumor in his mouth appeared largely gone; he was able to eat soup and solid foods, and begin returning to normal activities (Fig. 2C). The third cycle was interrupted after 2 weeks by recurrent infections associated with the large amounts of tumor necrosis, but the patient recovered and graduated from high school at week 21. Pazopanib had been restarted at week 17, and bevacizumab was discontinued after week 18. He received two additional i.t. injections and three i.m. injections of Hiltonol between weeks 21 and 25 with continued decrease in facial tumor. By week 25, the facial, oral, and retro-orbital tumor appeared largely gone or inactive on positron emission tomography (PET), with mainly healing normal structures upon gross inspection (Figs. 1F and 3). The large cerebral mass also appeared necrotic on MRI and was negative on PET (Figs. 1C and 3). However, there was still significant mass effect and, unfortunately, decompression craniotomy was postponed. The patient died suddenly with cardiorespiratory arrest at week 27, while being discharged from a brief hospitalization for the treatment of an infection. Cerebral herniation or pulmonary embolus was suspected, but autopsy was declined.

Discussion and Hypothesis

Immunotherapy is emerging as a major breakthrough in cancer management, concomitant with an increased understanding of basic immunology, tumor evasions and checkpoints, and recognition of the importance of the "danger signal" provided by PAMPs (1). Most cancer vaccines use one or more exogenous tumor-associated antigens, but the "host-targeted" strategy described here attempts to immunize against the patient's own tumor antigens in situ, and is not only theoretically applicable to various histologic tumor types but may also generate a more effective "natural" response to those antigens. Although the present case was complicated by the exceptional size of the tumor, intercurrent infections, and other treatments, it nevertheless provides encouraging evidence of the potential for relatively simple, safe, and inexpensive immunotherapies. However, the patient’s eventual death due to
suspected cerebral herniation and despite apparent necrosis of the exceptionally large cerebral lesion is a cautionary note about management of such cases.

Poly-ICLC (Hiltonol) is a "reliable and authentic" viral mimic and PAMP that at low doses in humans upregulates several hundred genes closely paralleling the pattern induced by a yellow fever live-virus vaccine, and representing some 10 canonical innate immune pathways (2–4). This includes the induction of a "natural mix" of IFNs, other cytokines and chemokines, activation of natural killer (NK) cells, T cells, myeloid dendritic cells (mDC) via melanoma differentiation-associated protein 5 (MDA5), TLR3, retinoic acid-inducible gene 1 (RIG-I) helicase, 2′,5′-oligoadenylate synthetase (OAS), the P68 protein kinase (PKR), and other dsRNA-dependent host defense systems, culminating in a broad innate antitumor and antiviral effect as well as a potent adjuvant effect, with inflammasome and other signaling (5). Poly-ICLC, thus, provides the dsRNA "danger signal" normally furnished by viral replication but that has been missing from many modern vaccines for cancer, HIV, malaria, and other diseases. In other words, when properly paired with antigens, poly-ICLC can generate a "live-virus vaccine-equivalent" with a comprehensive immune response that includes activation of mDCs and NK cells, processing of relevant antigens, generation of a polyfunctional Th1- and CTL-weighted response, homing to tumor or pathogen via induction of specific chemokines, and facilitation of CTL function through induction of costimulators such as OX40 (6–8). Importantly, the role of dsRNAs, such as poly-ICLC, may be bimodal, beginning with induction of IFNs and of dsRNA-dependent systems, and followed by their secondary activation by the dsRNA. Thus, two or more doses of poly-ICLC at a 24- to 48-hour interval or pretreatment with IFNs can markedly boost antiviral and antitumor activity, perhaps by better mimicking a natural infection (9, 10). This dosing regimen has also been used successfully in clinical glioma and in preclinical antiviral studies, as well as in the strategy presented here (5, 11–13).

Variations on the "autovaccination" strategy have included the combination of radiotherapy or chemotherapy to induce antigen release, with immunostimulants or oncolytic virus to induce an immune response (14–18). This approach is based on the hypothesis that i.t. administration of a PAMP will reverse DC inhibition in the treated tumor microenvironment, increase the efficiency of antigen presentation to CTLs, and prevent tolerization to tumor antigens. Such tolerization can be a counterproductive consequence of traditional chemotherapy or radiotherapy that induces release of tumor-associated antigens in the absence of a proper "danger signal" (14).

We postulate that the strategy presented here involves a three-step process. First, i.t. Hiltonol activates NK cells, various cytokines, TLR3, and other proapoptotic mechanisms, resulting in early tumor killing and antigen release (19–21). This may be the mechanism that led to the early local necrosis in the case reported here. This necrosis was observed before embolization or treatment with bevacizumab, although the latter may have contributed to the subsequent immune response. Second, in the same local context, Hiltonol recruits and activates mDCs and macrophages at the tumor site, in which they acquire tumor antigens that are released, present them to CD4⁺ helper cells, and cross-present them to CD8⁺ T cells in the tumor or in

Figure 2. A–D, patient 6 weeks pretreatment and at posttreatment weeks 2, 7, and 21.

Figure 3. PET at week 24 showing decreased uptake in both facial and intracranial tumor. Marginal uptake could represent active tumor or inflammation.
the regional lymph nodes to generate antigen-specific CTLs. 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 has also been used successfully with IT CpG and may be critical to this step (15, 17, 22). Timed release or structured formulations of poly-ICLC could even better mimic a viral infection, especially for deep tumors in which repeated i.t. administration is logistically more challenging. At the same time, the possibility of overstimulation with PAMP must be considered, and the optimal dose and schedule remain uncertain.

The third step is remote targeting and maintenance of antigen-specific CTLs via various poly-ICLC–induced chemokines, costimulatory factors, and other mechanisms (8). This activity directed at the local tumor microenvironment was perhaps illustrated by the local tumor inflammation in response to each remote i.m. injection of poly-ICLC in the reported case. Such inflammation with a gradual tumor response is typical of some immunotherapies (23). Murine sarcoma and lymphoma studies have demonstrated similar activity of IT poly-ICLC or the TLR9 agonist CpG, respectively, including at remote metastases, and especially when combined with anti–CTLA-4 and agonist anti-OX40, which boost T-cell responses (15, 17, 24). Other studies have demonstrated that poly-IC alone is sufficient to induce expression of the costimulatory molecules B7-H2, CD40, and OX40 (25). OX40 in particular may downregulate CTLA-4, decrease tumor-specific regulatory T cells (Treg), increase the T-effector/Treg ratio, and enhance CTL action in the tumor and metastases, and particularly with IT injection of a single target lesion (22, 26, 27). The dsRNA-dependent PKR induced by poly-ICLC will also activate the inflammasome pathway (2, 28). We, thus, expect that continued administration of poly-ICLC i.m. after optimal priming will facilitate and maintain sensitized CTL killing in the tumor microenvironment of both the injected and remote metastatic lesions. Clinical support for the concept of post-prime maintenance with i.m. poly-ICLC or IT, CpG alone also comes from recent successful glioma, lymphoma, and hepatoma trials (13, 17; A. de la Torre; unpublished data).

Exogenous bevacizumab, OX40, anti–CTLA-4, anti–PD-L1, FLT3L, and other costimulatory factors could further synergize with poly-ICLC and enhance this effect. Finally, poly-ICLC boosting can also enhance memory cell longevity (29).

A multcenter adaptive design two-phase II clinical study has begun in patients with the following advanced, unresectable metastatic solid tumors: sarcomas, melanoma, squamous cell skin cancer, and head and neck cancers (clinicaltrials.gov, NCT01984892). In each treatment cycle, one accessible tumor site is targeted for IT injections of 1 mg of Hiltonol three times a week for 2 weeks, followed by i.m. Hiltonol boosters twice weekly for 6 weeks. A second cycle is followed by a 6-week no-treatment period with evaluation of response at week 26, in the relative absence of inflammation. The primary endpoint is disease control at 26 weeks, as defined by the Immune-Related Response Criteria (23). Secondary objectives are overall survival and immunologic response in blood and tissue biopsies, including immunoscore.

In summary, we present results from a complicated case suggesting that sequential IT plus i.m. Hiltonol (poly-ICLC) can have a dramatic antitumor effect. We hypothesize that this in situ autovaccination strategy comprises three immunomodulatory steps. The first step is the innate immune local tumor killing induced by IT Hiltonol. A close second step is Th1- and CTL-weighted priming through the repeated in situ combination of the Hiltonol danger signal with the tumor antigens released in the first step and further processed and cross-presented by Hiltonol-activated mDCs. The third step is maintenance of the systemic antitumor immune response and migration of antitumor CTLs to remote metastases, through repeated IT Hiltonol induction of chemokines, OX40, and other costimulatory factors, as well as inflammasome activation. Exogenous costimulators or checkpoint blockers may enhance this action. An ongoing clinical trial in patients with solid cancers will further clarify these findings.

Disclosure of Potential Conflicts of Interest
A. Mark has ownership interest (including patents) and is a consultant/advisory board member of Oncovir, Inc. No potential conflicts of interest were disclosed by the other authors.

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References
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