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Canine Parvovirus-based Immunotherapy in Breast Cancer Patients, as Coadjuvant Treatment to Surgery Management: 9 Years Survival Following

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Authors' contributions

This work was carried out in collaboration between all authors. Author HRSP designed the study, wrote the protocol and wrote the first draft of the manuscript. Author JSSC managed the analyses of the study. Authors ITGM and JSSC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Breast Cancer continues to be a major oncological problem. Despite the several advances in medical science focusing on controlling this condition and avoiding relapse, is inevitable. The relapse rates with conventional treatment (surgery, chemotherapy and radiotherapy) continue to be high. Contributory treatment alternatives have emerged such as immunotherapy using an oncological virus which, *in vitro*, has proven effective with adequate security rates and with no relevant adverse side effects. Canine parvovirus (CPV), has shown a favorable security profile, induction of strong cytotoxic response, and oncosuppressing effects.
Methods: A clinical immunotherapeutic trial with the biological vaccine CIMT-54, approved by the Ethical Committee at the Manuela Beltrán University, on February 22nd 2007. We enrolled eight breast cancer patients who underwent surgery with their treating oncologic group, declined

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chemotherapy and radiotherapy, and started immunotherapy, with clinical and laboratory follow up for 9 years to the present.

Statement of Significance: Cancer continues to be a therapeutic challenge, despite reports on treatment that appear in the medical research literature. Breast cancer patients, have high rates of morbidity and mortality. The use of immunotherapy in cancer has grown in relevance during the last 20 years. Specifically, the use of a human parvovirus has facilitated the construction of a treatment profile with minimal negative side effects, being the only DNA virus with no tumorigenic members, so that it has cytopathic effects on abnormal cells and oncosupressing properties.

Keywords: Immunotherapy; canine parvovirus; breast cancer; virus-based vaccine.

1. INTRODUCTION

Immunotherapy has been to treat cancer since the mid twentieth century, initially as an aleatory response found in leukemia patients, for whom influenza infection seem to create a longer state of remission; nevertheless, few follow-ups were performed and the process was left unexplored. Two decades ago, immunotherapy once again began to gain importance as a contributory method to conventional cancer treatment [1]. Virotherapy is the field that evaluates viral effectiveness and has managed to define two large groups of viruses: 1) viruses with defective replicating vectors and 2) oncologic viruses competent in replication, which, in general terms, seek to induce immune response against tumoral cells, while preserving healthy cells [2]. The canine parvovirus (CPV) is a subgroup in the *Parvoviridae* family and a *Protoparvovirus* subtype that performs an oncolytic function derived from the presence of its capsid structures (allowing 90% infectivity contributed by VP2) as well as from phosphoprotein NS1. This phosphoprotein allows viral regulation and replication, genic expression, induction of cellular arrest that leads to tumoral cell apoptosis given the over expression of phosphokinases (PKC), induction of Reactive Oxygen Species (ROS) and, lately, it has been involved in the role of NS1 in the induction of DAMPs (Damage - associated molecular patterns) and PAMPs (Pathogen – associated molecular patterns) to generate antigenic presentation via TLR (Toll – like receptors) (specially TLR-3 and TLR-9) [3-9]. Biologically the use of viral immunotherapy (particularly the use of canine parvovirus) lies in the fact that the viral infection is, in general, asymptomatic for humans allowing a wider range of clinical security while being able to generate a robust immune response [5]. There are *in vitro* and *in vivo* (mice) approaches for using parvoviruses to treat tumorous processes, such as pancreatic and breast cancer, melanoma, and glioma [10-15]; however, this approach has not be extended to *in vivo* humans treatment (with the exception of Protocol ParvOryx in 18 patients with multiform Glioblastoma phase I/IIa, stage IV, comparing intratumoral/ intracerebral therapy vs. intravenous/intracerebral therapy [16]).

We will show clinical follow-up of breast cancer patients through the last 9 years until present, with complete survival and, in one case with a metastatic local- regional process, and in another case, axillar ganglionar metastasis, attributable to the absence of surgical treatment along with the absence of reinforcement doses of the CIMT-54 vaccine. The objective of this study was evaluate the survival rates, and evidence of disease in the patients under this vaccination protocol for a 9 year period.

2. MATERIALS AND METHODS

Our research team has been working for more than 20 years in the study of cancer immunotherapy, identifying the contributory therapy of canine parvoviruses in patients with diverse types of tumors; in this study, 8 cases of patients with breast carcinoma who declined conventional treatment with chemotherapy and radiotherapy, so that they only received radical surgery and, subsequently, consolidation immunotherapy using Canine Parvovirus.

Previous research *in vitro* and *in vivo* with Wistar rats demonstrated the research product's safety and efficacy being without any side effects (article in press). The research protocol "Clinical-Therapeutic Protocol of the Biologic Vaccine CIMT-54 against Cancer" was approved by the Ethical Committee of Manuela Beltran University and conducted under accord of February 22nd 2007, which includes the declaration of Helsinki, Article 37.

An announcement was made in the media (TV and radio) about the type of research the University was conducting on immunotherapy,

Table 1. Inclusion and exclusion criteria

Inclusion criteria
A. Patients who began immunotherapy treatment and rejected the use of conventional treatments as Chemotherapy, Radiotherapy
B. Patients with histopathotologic confirmation of cancer, established by a pathologist
C. Karnofsky index >69%
D. Life expectancy > 3 months
E. Informed consent approving receipt of immunotherapy scheme, and follow-up determinated by the protocol
F. Participation in an active Healthcare service (as the Colombia government laws indicate)
Exclusion criteria
A. Previous use of Radiotherapy, Chemotherapy or another experimental treatments
B. Seroposivity for HCV (Hepatitis C Virus), HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus)
C. Pregnancy or breastfeeding
D. Refusal to submit informed consent, or refusal of immunotherapy
E. Any medic, surgical or psychiatric condition that can interfere with completion of this study

with a specific call for breast cancer patients, due to the data found on the literature about the topic. A group of patients was selected based on Inclusion and Exclusion criteria (Table 1 above), and considering the legal and ethical implications placed on the subjects of research. This study was funded by Manuela Beltran University.

The study was conducted on eight previously diagnosed cases of breast carcinoma ranging in ages from 30 - 74 who entered the research protocol in the Cancer Research Laboratory in 2007, including seven cases that were treated with mastectomy or quadrantectomy and immunotherapy using CIMT-54 biological vaccine, and one case of that received only immunotherapy without surgical treatment. Patients were entered into the study for 9 months after the committee approval (February 22/2007). The monitoring of each one of the cases included the patient self-reporting, physical exam, ultrasounds, mammographies, scintigraphies and blood counts; Clinical follow-up was every month, and paraclinicals follow-up was every 3 months. None of the patients participating in this study has died. The collection of information was obtained from clinical histories. The Canine Parvovirus used was obtained from Virbac Laboratory (Bogotá, Colombia), under the commercial name PARVIGEN.

3. RESULTS AND DISCUSSION

In the evaluated period 2007-2015 we conducted the clinical follow-up of eight cases with a diagnosis of breast cancer who received immunotherapy with the biological vaccine CIMT-54 against cancer. 87.5% of the monitored cases exhibited infiltrating ductal carcinoma and the rest exhibited lobulillar type; most diagnosed with cancer after the age of 50. Only 12.5% were negative for the expression of estrogen and progestogen receptors, and the rest of the cases showed positive expression > 80% (Table 2).

Table 2. General characteristics of the study

Charateristics	Classification	No (%)
Carcinoma	Infiltrating ductal	7 (87.5)
	Lobulillar	1 (12.5)
Age	Less than 50 years	3 (37.5)
	Older than 50 years	5 (62.5)
Estrogen receptors	Positives	6 (75)
	Negatives	1 (12.5)
	No information	1 (25)
Progestogen receptors	Positives	6 (75)
	Negatives	1 (12.5)
	No information	1 (12.5)
Pathological scale in the Bloom and Richardson scale.	II	3 (37.5)
	II/III	4 (50)
	III	1 (12.5)
BIRADS classifications prior to treatment.	4	3 (37.5)
	5	2 (25)
	6	1 (12.5)
	No information	2 (25)
Surgery	Mastectomy	3 (37.5)
	Quadrantectomy	4 (50)
	No surgery	1 (12.5)

Most of the evaluated cases had between 2 and 5 mammographies after immunotherapy during the evaluated period, except for patient 5 (who had no surgery, but received immunotherapy). Her evolution was monitored with other imaging exams (thorax CT and ultrasounds). In the thorax CT of this patient, there were no masses in the pulmonary parenchyma, just the mass of speculated contours corresponding to carcinoma. In her last ultrasound, performed in 2014, a nodular solid lesion was observed with a classification of Bi RADS 5 corresponding to carcinoma. Patient 6 exhibited benign signs in

mammographies until 2011, but in 2014 had a biopsy showing canicular infiltrating carcinoma with a classic moderately differentiated nuclear degree 2 and a carcinoma ductal component, leading to a radical mastectomy the same year. A local regional recurrence was detected seven years after immunotherapy application. None of the patients evaluated has died (Table 3).

The results of the blood count of each patient following the start of the immunotherapy was within the normal ranges (Table 4).

Table 3. Case description

Cases	Surgery	ER/PR	TNM	PTM	NPTM	PTS	NPTS	LRR	Metastasis
1	Mastectomy	P/P	IIA	4	4	1	1	No	No
2	Quadrantectomy	P/P	IA	5	5	4	4	No	No
3	Mastectomy	P/P	IIA	3	3	2	2	No	No
4	Mastectomy	N/N	IIB	3	3	2	2	No	No
5	No	P/P	IIB	0	0	2	2	No	Yes
6	Quadrantectomy	No data	IA	3	3	3	3	Yes	No
7	Quadrantectomy	P/P	IA	2	2	1	1	No	No
8	Quadrantectomy	P/P	IIIB	3	3	3	3	No	No

ER/PR: Estrogen/Progestagen Receptors, PTM: Post Treatment Mammographies, TNM: Tumor Node Metastasis Breast Cancer Staging NPTM: Negative Post Treatment Mammographies, PTS: Post Treatment Scintigraphies, NPTS: Negative Post Treatment Scintigraphies, LRR: Loco-Regional Recurrences

Table 4. Results of the blood count in the evaluated period

Case	Parameter	Pr.I	1M PI	1Y-2Y PI	6Y-8Y PI
1	Hb (g/dl)	14.6	14.2	14.8	14.70
	Hto (%)	45.8	43.9	47	43.8
	Leuco. x 10^9 /L	7.12	5.49	7.30	5.61
	Neu (%)	65.4	58.5	52.1	60.60
	Lyn (%)	27.9	32.8	40.2	28.70
	Plt x 10^9 /L	323	248	326	274
2	Hb (g/dl)	13	14.3	15	14.9
	Hto (%)	38.3	41.9	44.4	44.6
	Leuco. x 10^9 /L	8.62	9.28	7.62	8.61
	Neu (%)	73.2	66.5	58	54.10
	Lyn (%)	20.5	25.9	37	37.20
	Plt x 10^9 /L	373	532	215	261
3	Hb (g/dl)	14.2	13.5	13.9	14.1
	Hto (%)	44	41	43	44.2
	Leuco. x 10^9 /L	6.2	4.8	5.4	7.84
	Neu (%)	53.3	53	63.4	66
	Lyn (%)	35.5	46	26.8	23.7
	Plt x 10^9 /L	319	ND	278	316
4	Hb (g/dl)	14.40	14.4	14.7	13.9
	Hto (%)	43.00	43	42.5	42.4
	Leuco. x 10^9 /L	5.3	4.8	4.16	4.36
	Neu (%)	52	42	63.2	58.70
	Lyn (%)	48	58	33	36.50
	Plt x 10^9 /L	ND	ND	380	286
5	Hb (g/dl)	16.70	16	15.7	16.2
	Hto (%)	44.00	47.4	45.1	46.6
	Leuco. x 10^9 /L	8.37	7.3	8.25	7.14
	Neu (%)	50.90	49	64.3	50.3
	Lyn (%)	37.90	41.7	26.5	41.6
	Plt x 10^9 /L	260	270	285	271

Case	Parameter	Pr.I	1M PI	1Y-2Y PI	6Y-8Y PI
6	Hb (g/dl)	15.60	ND	ND	14.4
	Hto (%)	46.10	ND	ND	41
	Leuco. x 10^9 /L	8.6	ND	ND	7.4
	Neu (%)	67	ND	ND	58.2
	Lyn (%)	28	ND	ND	33.3
	Plt x 10^9 /L	227	ND	ND	237
7	Hb (g/dl)	13.6	13.2	13.9	12.7
	Hto (%)	42	40	40.7	40.7
	Leuco. x 10^9 /L	4.58	4.4	5.3	4.53
	Neu (%)	54.6	46	47.6	2.42
	Lyn (%)	34.9	51	48.6	34.7
	Plt x 10^9 /L	221	ND	257	239
8	Hb (g/dl)	12.8	13	14.7	14
	Hto (%)	39.1	39	42.1	42.5
	Leuco. x 10^9 /L	7.48	7	7.24	7.77
	Neu (%)	56	66	57.2	52
	Lyn (%)	44	34	21.3	22
	Plt x 10^9 /L	343	249	210	144

Pr.I: Prior to immunotherapy, 1M PI: 1 month post immunotherapy, 1Y-2Y PI: 1 to 2 years post immunotherapy, 6Y-8Y PI: 6 to 8 years post immunotherapy, ND: No data

4. CONCLUSION

The National Institute of Cancer reported that during 2007 to 2011 in Colombia there were 62818 new cases of cancer, of which 29734 (47.33%) were men and 33084 (52.66%) women, with 32653 deaths per year, 16081 (49.24%) in men and 16572 (50.75%) in women. During the same period, there were 7,627 (23%) cases of breast cancer, with a relative frequency of 12.3% compared with other types of cancer, and a death rate of 9.3 deaths per 100,000 inhabitants, resulting in a public health problem as the most prevalent cancer type in women [17,18]. In 2007, for the study "Clinical-therapeutic Protocol for the CIMT-54 biological vaccine against cancer" eight female patients were enrolled who had been diagnosed with invasive ductal carcinoma previously treated with mastectomy (3), quadrantectomy (4), and one (1) patient without surgical treatment. They all received clinical follow-up for nine years. All the patients were treated with 6 doses of the CIMT-54 biological vaccine. None of them died during the test period and only one patient developed loco-regional recurrence (attributable to the lack of surgical therapy). Our results differ from those published in a study carried out in 2010 in Colombia, in which a 6.3% of recurrence was detected in the cases following mastectomy and radiotherapy, with the average time to recurrence being 1.82 years. The accumulated 5 year survival without loco-regional recurrence was 88.8% up to 5 years. Of the patients who had recurrences, 45.5% had them in the prior two years following the radiotherapy [19].

In the study done by Ospino and collaborators, 63.6% of the patients had recurrences at multiples s, of which the ipsilateral supraclavicular fossa was the most prevalent. In our study, bone scintigrapiteshies performed during the nine years of follow-up did not showed any signs of malignancy or metastasis [19].

Current research is focused on developing new tools for improved cancer treatment, including the reduction of different adverse side-effects. Researchers have been working on the utility of immunotherapy, as it seeks to enhance the patient's quality of life and to lessen the disease's progression after reactivating the immune system against tumor antigens, reversing immunotolerance induced by cancer and generating the antitumoral immune response. The aim of immunotherapy lies in identifying the appropriate specific and adjuvant-tumor antigens necessary to stimulate the dendritic cells and afterwards an optimal activation of the T cells [20]. The treatment used in the present study consists of an immunological protein complex that initially stimulates an immunological cell response mediated by the Natural Killer (NK), monocyte and macrophage cells. This recruitment encourages the production of IFN gamma, which allows the activation of macrophages and induces an adaptive

immunological response mediated by the T- and B-lymphocytes, that promotes lymphocytic infiltration of the tumour. In studies carried out by Raykov and collaborators in 2007, the oncolytic virus induced oncolysis that affected pancreatic tumour cells; this was reaffirmed in 2011 by Bhat and collaborators, who showed that the virus leads to a lessening of CMH-I in the Panc-1 cells, causing the cells to be susceptible to the recognition of the NK and CD+8 cells, that is due to the increase in the activation of the ligands of the NK cells and a decrease in the CMH-I expression [21,22]. Additionally, the CIMT-54 vaccine includes the tumor antigen, as seen in studies such as Moehler's 2005 study which showed that the dendritic cells co-cultivated with cells infected with the oncolytic virus encourage the presentation of the antigen associated with the tumor and its phagocytosis resulting in an effective activation of the antitumor specific response [11].

Research in immunotherapy have been carried out for cancer management, including the 2007 study in the Hospital de Viedma in Bolivia where the case of a 58-year-old man diagnosed with superficial papillary carcinoma of the bladder, received immunotherapy with BCG, via bladder instillation. Improvement in patient status were observed and within a year tumor recurrence on the bladder's surface was absent. After three years, a bladder mass was detected and it's anatomopathological report identified a stage IA transitional papillary carcinoma. Therefore, the patient received additional immunotherapy that resulted in of improvement in the patient. However, following the immunotherapy the patient developed tuberculous cystitis as a complication.

In the present study, the patients had a 9-year evaluation period unlike the study previously mentioned which was only for 4 years. The immunotherapy with CIMT-54 has shown that the patients achieved a good state of health based on their clinical lab results, anamnesis and diagnostic imaging, and they have not shown any side-effects to the vaccine, in contrast to the Bolivian study in which tuberculous cystitis developed. Finally, we employed subcutaneous application in our patients, instead of application to the tumor, which is less traumatic for the patient, and less prone to bacterial infection as occurred in the bladder instillation [23].

Roychoudhuri and collaborators in 2007, reported an increase in cardiovascular mortality induced by radiotherapy, in women with breast cancer at 50 years old and who live to the age of 65. Without radiotherapy, they have a 25% risk of developing cardiovascular disease, and if they receive radiotherapy, the risk is increased to 30% [24]. In the present study, radiotherapy was not used in order to avoid the side-effects caused by the radiation in the patient. The approach presented here was developed in orde to avoid cardiovascular damage from radiotherapy and demonstrated no signs of cardiovascular disease after 9 years of evaluation.

The risk of recurrence, based on previous studies, is two times higher in young women than in women older than 50, and it can increase if treatment with radiotherapy is not done, which would be reflected in a higher death rate due to breast cancer. The onset of local recurrences has a negative impact on the patient's diagnosis, since it facilitates the metastasis process, allowing the development of a local cancer or a new primary cancer [19,25]. In the current study, based on the analysis of lab results and diagnostic imaging one of the cases who was less than 50 years old has not shown any signs of malignancy or metastasis after. Her post quadrantectomy treatment exclusively consisting of CIMT-54 biological vaccine.

During follow-up, neither kidney nor liver function was affected by the immunotherapy since the levels of creatinine and the liver enzymes were in the normal ranges. The level of CA15-3 was examined during each patient's follow-up and it did not show an increase indicating an adequate response to the treatment and stability of the disease. As opposed to other type of treatments which can lead to anaemia and leukopenia, the CIMT-54 biological vaccine did not result in any side-effects in the patients over the 9-year evaluation period based on the follow-up of the blood count during this period.

This study is the first case-report of breast cancer cases treated with immunotherapy in Colombia, and shows the effectiveness over 9 years showing side-effects. Only in one case was there the presence of loco-regional metastasis (the female patient without surgical adjuvant therapy, who did no receive reinforcement doses of the vaccine.) A 100% overall survival rate was observed during the follow-up of 9 years accounting for disease-free survival. At present, molecular approaches are being developed to have much greater focus on the signalling pathways and checkpoints where the CIMT-54

vaccine has its influence, and as such hold even greater promise.

DISCLAIMER

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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