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INVESTIGATOR BROCHURE

"AUTOLOGOUS DENDRITIC CELL LOADED WITH AUTOLOGOUS TUMOR HOMOGENATE OR LYSATE VACCINE"

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LIST OF ABBREVIATIONS

AE	Adverse Event
APC	Antigen presenting cell
CEA	Carcinoembryonic antigen
CRC	Colorectal cancer
DC	Dendritic cell
DTH	Delayed type hypersensitivity
GM-CSF	Granulocyte Monocyte Growth Factor
IFN-γ	Interferon γ
IL1β	Interleukin 1β
IL2	Interleukin 2
IL-4	Interleukin 4
IL6	Interleukin 6
IMPD	Investigational Medicinal Product Dossier
KLH	Keyhole limpet hemocyanin
OS	Overall survival
PGE2	Prostaglandin E2
PR	Partial response
SAE	Serious Adverse Event
SD	Stable disease
Th1	T Helper 1
TNFα	Tumor Necrosis factor α

CHEMICAL PHARMACEUTICAL AND BIOLOGICAL DATA

1. SUMMARY

1.1 Introduction

This "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" is an Advanced Therapy Medicinal Product consisting of dendritic cells obtained by in vitro differentiation of peripheral blood monocytes, isolated by leukapheresis from each patient, with IL-4 and GM-CSF. Immature DC such obtained are then loaded with an homogenate or lysate of tumor tissue obtained from the same patient, matured with a cytokine cocktail containing IL1β, PGE2, IL6, and TNF-α ("maturation cocktail"), and then intradermally administered; see figure 1.

Dendritic cells are widely distributed antigen-presenting cells playing a central role in the activation and regulation of immune response.[1] It is largely established that tumor cells produce several biologically active substances which strongly influence the ability of DC to prime and sustain effective immune responses.[2,3] Ex-vivo reconditioning of dendritic cells, together their loading with tumor-derived antigens has been largely utilized in patients carrying malignant tumors of several type and origin since its first clinical utilization in melanoma patients in 1996.[4]

In this respect, dendritic cells (DC) loaded with tumor antigens, differentiated and matured *in vitro* have been largely shown to efficiently induce and/or potentiate tumor-specific immune responses which underlay their anticancer activity.[5,6]

1.2 Non-clinical studies

A very large series of observations in preclinical murine models extensively demonstrated that dendritic cells are potent antigen-presenting cells (APCs) which can mediate regression of advanced tumors after pulsing with tumor-derived antigens and adoptive transfer in tumors of different origin and type, melanoma in particular (reviewed in Kochenderfer et al).[7] Dendritic cell vaccines activity in preclinical models is related to their ability to induce tumor-specific T cell responses with a prevalent Th1 profile.[8]

Moreover, preclinical data showed that human monocyte-derived dendritic cells obtained with the same differentiation and maturation protocols utilized for the production of the "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" efficiently induce antigen-specific IFN-

γ -producing CD4 and CD8 T cells in vitro.[9]

Given the availability of extensive preclinical and clinical characterization of dendritic cell obtained with the same protocol utilized for manufacturing the "autologous dendritic cell loaded with autologous tumor homogenate of lysate vaccine" described in this Investigator Brochure no additional preclinical data have been provided.

1.3 Effects in humans

Dendritic cell vaccines typically lack significant toxicity; data obtained from the first 1000 DC vaccines mainly reported the occurrence of local reactions (erythema and induration in the injection site, regional reactive adenopathy), fever and/or fatigue during treatment, with no hospitalizations nor death attributed to the administration of DC vaccines.10

Monocyte-derived dendritic cells obtained according to the same protocol utilized for the manufacturing of the "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" have been employed in patients with advanced melanoma since 2001.11-15

In most studies above cited monocyte-derived DC were well tolerated, with AE mainly limited to local reactions (swelling and pain) in the injection site and/or in locoregional lymphnodes and fever. A few grade III-IV AEs were observed only in one of these studies.13

A limited number of clinical studies employed monocyte-derived DC obtained with IL4 + GM-CSF, loaded with autologous tumor lysate and matured with IL1β, PGE2, IL6, and TNF-α, (comparable to the medicinal product "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" herein described).

In particular, Hersey et al reported a clinical benefit (CR+PR+SD) of 18.75% in 16 advanced melanoma patients treated in a phase I/II clinicl studies.11

Clinical efficacy

Since 2001, in a phase I/II open label, single arm clinical study we have treated patients carrying advanced melanoma (stage IV or non-resectable stage III) with autologous DC loaded with autologous tumor lysate or homogenate and matured with a cytokine cocktail, observing a clinical benefit (CR + PR + SD) in 55.5% of the 27 evaluable patients and a median overall survival of 16 months (95% CI: 13.4-61.3).16-18

In this clinical study, patients which developed antitumor immunity after vaccination experienced a

better clinical outcome, confirming other groups' experience,19,20. in particular, we observed that the 19 patients which develop Delayed Type Hypersensitivity (DTH) against autologous tumor lysate or Key-Hole LympetHemocyanin (KLH) after at least 4 courses of the vaccine show a median overall survival (OS) of 22.9months compared with 4.8 months observed in the 8 DTH-negative cases (Log-rank test, p=0.007).

Clinical safety

Treatment-emergent adverse events (AEs) considered by the Investigator to be related to study drugs (DC vaccine or Interleukin-2) were reported for 47.6% (39/82) of all patients treated with the medicinal product "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" either in the phase I/II clinical study above described or under nominal request of the physician according to art.2 comma 1 lettera f of DM 5 dicembre 2006 ("Utilizzazione di medicinali per terapia genica e per terapia cellulare somatica al di fuori di sperimentazioni cliniche e norme transitorie per la produzione di detti medicinali"), table 1.

In Table 1 are also included drug-related side effects observed in the 24 patients enrolled in the ABSIDE study (EudraCT: 2012-001410-41) and in 10 patients enrolled in COREVAX study (EudraCT: 2015-000894-11) ongoing at our institute. Among these patients, most frequently side effects included erythema, pruritus at vaccine injection site, and maculopapular skin rash and fever after low dose IL-2 administration. To date we observed no grade 3-4 toxities in the ABSIDE study neither in the Corevax one.

Any Drug-related AE	Nr of subjects	%	Nr from ABSIDE (%)	Nr From Corevax (%)
Grade 1	26	31.7	18 (75%)	8 (80%)
Grade 2	9	11	6 (25%)	2 (20%)
Grade 3	4	4.9	0	0
Grade 4	0	0	0	0
Grade 5	0	0	0	0
All grades	39	71.4	24 (100%)	10 (100%)
Anyserious AE				
Grade 3-4	8	9.8	0	
Any Drug-related serious AE				
Grade 3-4	4	4.9	0	

Table 1. Summary of on-study adverse events

2. INTRODUCTION

"Autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" is an advanced therapy medicinal product consisting of autologous monocyte-derived dendritic cells obtained upon differentiation with GM-CSF and IL4, loaded with autologous tumor lysate or homogenate, and matured with a maturation cytokine cocktail consisting of IL1β, PGE2, IL6, and TNF-α.

Dendritic cells are widely distributed antigen-presenting cells that play a central role in the activation and regulation of immune response.1

In particular, DC determine the final outcome of adaptive immune responses against a specific antigen, i.e. whether it will be tolerized or a specific immune response must be raised against. The final outcome depends on the balance between different signals acting on DC while uptaking antigens. Indeed, if immature DC recruited to peripheral tissues find appropriate "danger signals" (i.e. pathogens products able to trigger toll-like receptors) they undergo full maturation and migrate to the lymphnodes, where they initiate a specific immune response; conversely, if danger signals are not present, or if concurrent immunosuppressive stimuli occur, DCs do not mature or undergo incomplete maturation thus inducing immune tolerance against antigens they are presenting.

Along cancer progression tumor cells acquire the ability to either evade the immune response by the selection of lesser immunogenic variants ("cancer immunoediting")21,22 and/or by producing immunosuppressive cytokines and other biologically active substances which strongly influence the ability of DC to prime and sustain effective immune responses.23,24

2.1 Background

In 1996, Schadendorf's group firstly tested in melanoma patients the feasibility of a vaccination strategy aimed to recondition DC function by their differentiation and loading with tumor antigens ex vivo thus allowing to overcome the effects of a DC-tolerizing tumor microenvironment.4 Since this first experience, it has been estimated that over one thousand patients with different tumors have been treated, using different starting cells and differentiation/maturation protocols, as well as different antigen sources and administration routes.10

In agreement with the mechanism which underlie the clinical efficacy of DC vaccines in cancer patients, several observations from the literature strongly indicate that patients which develop antitumor immunity after vaccination experience a better clinical outcome. In particular, in a phase I/II clinical

study patients developing Delayed Type Hypersensitivity (DTH) against autologous tumor lysate or Key-Hole LympetHemocyanin (KLH) after at least 4 courses of the vaccine showed a median overall survival (OS) of 22.9 months compared with 4.8 months of DTH-negative cases (Log-rank test, p=0.007).[16]

3. PHYSICAL/CHEMICAL AND PHARMACEUTICAL PROPERTIES AND FORMULATION

3.1 Physical/chemical

Not applicable.

3.2 Pharmaceutical properties and formulation

3.2.1 Description of the dosage form

Each dose of the IMP contains 10x106autologous mature dendritic cells pulsed with autologous tumor homogenate, resuspended in 2 ml of 0.9% sterile saline to dilute the cells at the concentration of 5×106 cells/ml, and provided in 2 insulin syringes, ready for use.

3.2.2 Drug product preparation

Each vaccine dose is provided in 2 ready-to-use insulin syringes in a plastic bag provided with a label indicating the lot number, released by the IRCCS IRST Laboratory of Somatic Cell Therapy.

3.2.3 Recommended storage and use conditions

The dendritic cell vaccine formulated as live cell suspension in sterile saline provided in insulin syringes is released by the Laboratory of Somatic Cell Therapy IRCCS IRST Cell and sent to the ward where it is administered to patients. If not immediately administered, the product must be maintained at room temperature for 60'or at 2-8°C for 8 hours.

4. NON-CLINICAL STUDIES

Given the availability of extensive preclinical and clinical characterization of dendritic cell obtained with the same protocol utilized for manufacturing the "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" described in this Investigator brochure no additional preclinical data have been provided.

5. EFFECTS IN HUMANS

5.1 Clinical pharmacodynamics

Not applicable.

5.2 Clinical pharmacokinetics

In vivo migration study performed by labeling monocyte-derived DC with 99mTc-HMPAO and 111In-Oxinein 5 patients treated with the "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" showed that DC intradermally administered efficiently migrate to locoregional lymphnodes. In particular, labeled DC can be detected in lymphnodes within 60 minutes after administration, with the peak reached after 18-20 hours.25

5.3 Plasma concentration-effect relationship

This cellular therapy exert its activity by migrating to lymphnodes after intradermal injection and by inducing antigen-specific immune response, not requiring distribution to the plasmatic compartment. Therefore, no data concerning plasma concentration-effect relationship are provided.

5.4 Clinical efficacy

Since 2001, in a phase I/II clinical study we have treated patients with advanced melanoma autologous DC loaded with autologous tumor lysate/homogenate and matured with a cytokine cocktail, observing a clinical benefit (PR + SD) in 55.5% of the 27 evaluable patients and a median overall survival of 16 months (95% CI: 13.4-61.3).16

In our 16-18 and other groups'experience, 19,20 patients which develop antitumor immunity after vaccination experienced a better clinical outcome: in particular, we observed that patients developing Delayed Type Hypersensitivity (DTH) against autologous tumor lysate or Key-Hole Lympet Hemocyanin (KLH) after at least 4 courses of the vaccine show a median overall survival (OS) of 22.9months compared with 4.8 months of DTH-negative cases (Log-rank test, p=0.007; figure 1).[16]

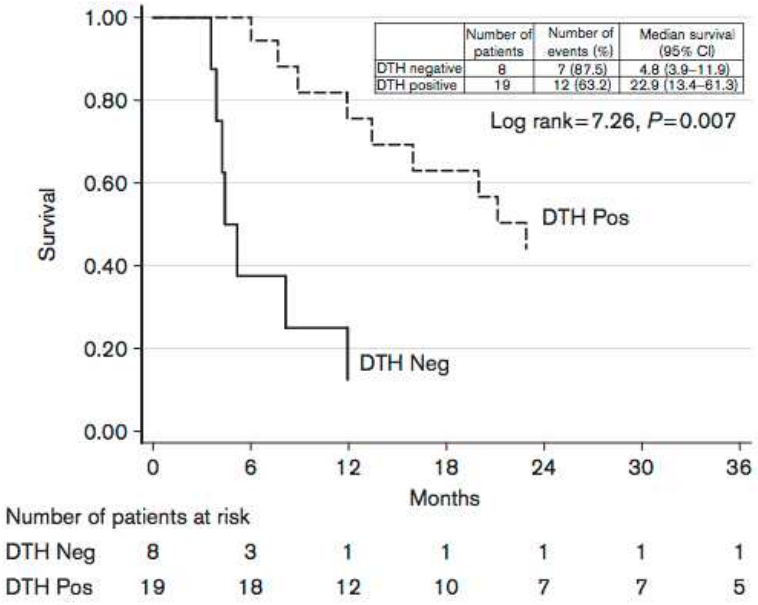

Figure 1. Survival analysis of the 27 immunologically evaluable patients according to the immunological response to the vaccine (phase I/II clinical study).

Analysis of clinical efficacy was extended to additional 55 patients that received the IMP herein described "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" under nominal request according to the same inclusion criteria and treatment schedule of the above described phase I/II clinical study. Extended analysis showed a clinical benefit (CR + PR + SD) in 49.3% (95% CI: 37.7-60.9) of the 71 evaluable patients (Table 2), a median overall survival of 12.01 months (95% CI: 8.06-16.74).

Best ClinicalResponse	Nr of subjects	%
Notevaluable	11	13.41
ProgressingDisease (PD)	36	43.9
StableDisease (SD)	31	37.8
PartialResponse (PR)	2	2.44
Complete Response (CR)	2	2.44

Table 2. Clinical response observed in the 82 patients treated with "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine"

Survival analysis was performed in the 54 patients for which Delayed Type Hypersensitivity (DTH) testing had been made, and showed that immunoresponsive patients (positive DTH against tumor lysate and/or KLH), have a median overall survival (OS) of 22.76months (95% CI: 15.16-36.09) compared with 8.06

months (95% CI: 5.23-12.43) of DTH-negative cases (Log-rank test, p=0.0036; median follow-up = 71 months; figure 2).

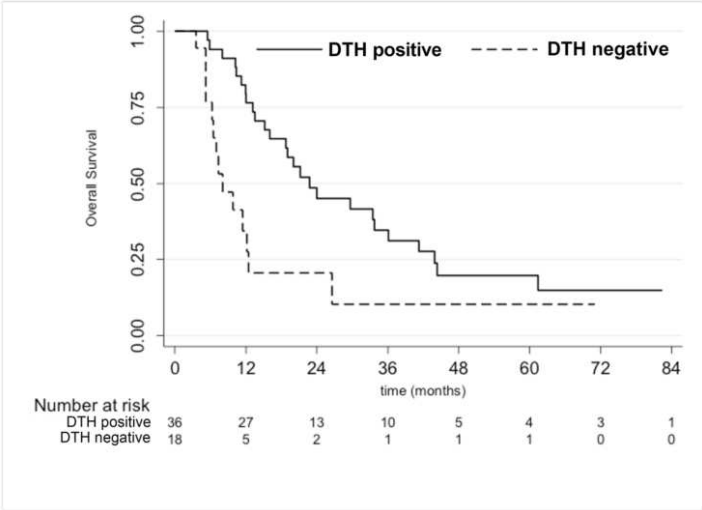

Figure 2. Survival analysis of the 54 immunologically evaluable patients according to the immunological response to the vaccine.

Similarly, immunoresponsive patients showed significantly longer progression-free survival than non-immunoresponsive ones with 6.81 months (95% CI: 5.03-10.16) vs 3.40 months (95% CI: 3.06-4.70), respectively; p = 0.0197; figure 3.

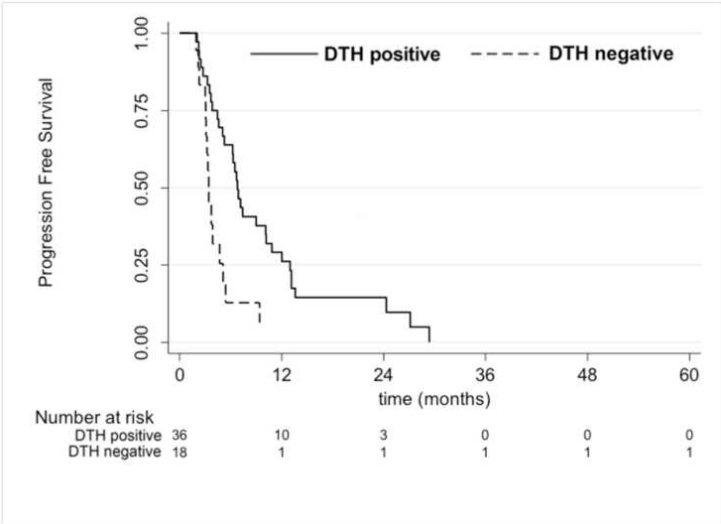

Figure 3. Progression-free survival analysis of the 54 immunologically evaluable patients according to the immunological response to the vaccine.

5.4.1 Clinical efficacy in colorectal cancer

This product has not specifically tested in CRC patients. However a very similar one, composed by DCs generated and matured with the same cytokine cocktail and pulsed with KLH and CEA peptides, was tested in two studies by another group.

The first study was conducted on resectable metastatic CRC patients who receive the vaccine as complementary treatment before and after surgery. Among the six patients who underwent surgery with radical intent only one relapse was observed26. The second one enrolled resected stage III CRC patients who were treated with the same product plus standard adjuvant chemotherapy with oxaliplatin and capecitabine. No relapses were observed among seven patients27.

Immunological efficacy was primarily assessed with DTH skin test. Concerning the first clinical trial, the single relapsed patient plus another one were classified as non-responders, while the other four patients were all immunological responders. In the second trial all patients became responsive to KLH and four to CEA after immunization.

Together, these data suggest that a DC vaccinecan induce cell-mediated immune responses potentially reducing relapse risk after radical surgery for CRC.

5.4.2 Clinical efficacy in High Grade Gliomas

Numerous preclinical studies have attempted to evaluate the efficacy and feasibility of DC vaccine in gliomas. One of the earliest studies of glioma immunization attempted to demonstrate that therapeutic immunization in established tumors is possible. Siesjo et al. showed that pre-immunization of mutagen-treated rat glioma N32 cells led to the rejection of subsequent subcutaneous injection and intracerebral implantation of weakly immunogenic unmutated N32 gliomas. The group subsequently demonstrated that immunization of weakly immunogenic unmutated tumor cells with adjuvants such as DCs led to significant therapeutic effects equivalent to the clinical benefits of immunization with mutated cell lines28. A similar experimental model using the 9L rat glioma cell line yielded similar results and showed the effectiveness of DCVs in cytotoxic CD8+ T cell-mediated anti-tumor immunity29. The authors demonstrated increased infiltration of CD8+ T cells in the TME as shown by immunohistochemistry (IHC) as well as increased in vitro 9L cell lysis by CTLs after vaccine treatment compared to the control group. Despite differences in techniques, these studies demonstrated the potential of DC vaccines to elicit anti-tumor response.

On HGGs (High-grade gliomas) multiple phase I/II trials have been reported; close to 500 patients with GBM have been treated with DC vaccination in more than 38 studies and all of these documented feasibility and safety30-32. Even if the objective response rate was only 15.6% two meta-analysis published in 201433 and some controlled studies indicated improved survival (OS) and progression free survival (PFS) with DC vaccination in HGGs patients34. In 16 non-randomized studies the median OS of newly diagnosed GBM patients ranged from 11.0 to 38.4 months. Moreover a systematic review by Wang X. of 171 studies confirmed an advantage for DC vaccination in terms of OS and PFS without severe adverse events (Ads) and despite of cycles, doses and route of administration35.

5.5 Clinical safety

Since the first experience in patients with metastatic melanoma in 1996,[4] vaccination with autologous dendritic cells loaded with tumor antigens has been largely shown to elicit tumor-specific immune responses potentially able to effectively kill cancer cells without inducing meaningful side effects.

5.5.1 Adverse events

Swelling, redness and pruritus around the site of injection of the vaccine, mostly G1-G2 and selflimiting, were the only toxicities observed in patients treated in our previous phase I/II clinical study.7-9

Extended analysis of safety encompassing 82 patients treated with the IMPs (DC vaccine and Interleukin-2) either in the phase I/II clinical study or under nominal request, showed that treatment-emergent adverse events (AEs) were reported for 38 of the 82 evaluable patients (46.34%) treated with the medicinal product "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" (table 3).

System Organ Class Preferred terms (CTCAE v.5.0)	G1	G2	G3	G4	Total
Anemia	0	1	2	0	3
Astenia	1	1	0	0	2
Epigastralgia	0	1	0	0	1
Eritema (injection site)	2	1	0	0	3
Fever	8	1	3	0	12
Flu-likesyndrome	1	0	0	0	1
Hypostenia	2	0	0	0	2
Induration (injection site)	12	1	0	0	13
Myalgia	0	1	0	0	1
Nausea	2	1	0	0	3
Pneumonitis (Aspergillusfumigatus)	0	1	0	0	1

Polymiositis	0	0	1	0	1
Pruritus (generalized)	0	1	0	0	1
Pruritus (injection site)	1	0	0	0	1
Tromboembolism	0	2	0	2	4
Urticarioidskinrash	4	1	0	0	5
Total	34	13	6	2	54

Table 3. On-study Adverse Events (AEs). AEs were categorized according to CTCAE v.5.0

System Organ Class Preferred Terms (CTCAE v.5.0)	Related to DC Vaccine	Related to IL-2	Related to IMPs	Total
Anemia	0	0	3	3
Astenia	1	1	0	2
Epigastralgia	0	1	0	1
Eritema (injection site)	2	1	0	3
Fever	0	12	0	12
Flu-likesyndrome	0	1	0	1
Hypostenia	0	2	0	2
Induration (injection site)	7	6	0	13
Myalgia	0	1	0	1
Nausea	0	0	3	3
Pneumonitis (Aspergillus fumigatus)	0	0	1	1
Polymiositis	0	1	0	1
Pruritus (generalized)	0	1	0	1
Pruritus (injection site)	1	0	0	1
Tromboembolism	0	0	4	4
Urtikarioidskinrush	0	5	0	5
Total	11	32	11	54

Table 4. INPs-related Advese Events (AEs). AEs were categorized according to CTCAE v.5.0

In these patients, most of the observed AE were related to IL-2, which is administered after each vaccine dose as immunological adjuvant. In particular, the most frequently reported treatment-related AEs of any grade included fever after IL-2 administration, erythema and pruritus at vaccine injection site, and maculopapular skin rash after IL-2 administration (table 4). Twentyfour additional patients enrolled in the ABSIDE study (EudraCT: 2012-001410-41) and 10 patients enrolled in COREVAX study (EudraCT: 2015-000894-11) ongoing at our institute were evaluated for the AE to date and among these, most frequently side effects included erythema, pruritus at vaccine injection site, and maculopapular skin rash and fever after low dose IL-2 administration.

Similarly, the studies on CRC patients showed a very favourable safety profile. In both clinical trials toxicity was limited to grade 1 or 2 adverse events, mostly flu-like symptoms. No grade 3 or higher

adverse events were reported[26-27]. In addition, other small clinical trials evaluated DC vaccines generated, loaded and matured with different techniques and antigens, and confirmed the excellent tolerability of the products: adverse events were limited to grade 1 or 2 injection site reactions, fever, chills and diarrhea[36-39]. These data support the good tolerability of DC vaccines also in this setting, so it is expected that the safety profile of the medicinal product "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine"be largely unchanged in CRC patients.

5.5.2 Serious adverse events

Only two G4 adverse events was observed in the 82 patients (2.4%) treated with the medicinal product "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine". However, these patients showed paucisymptomatic subsegmentary pulmonary embolism which was not likely related to the treatment. No grade 3-4 toxities in ABSIDE or COREVAX were registered to date.

5.5.3 Expected adverse events

Expected AEs and SAEs are presented by system organ class in Table 3. The listing of events is based on individual case reports in which at least one case of such an event has been attributed to the study therapy, although a true causal relationship cannot be fully determined. All AEs and SAEs not listed in Table 3 will be classified as Suspected Unexpected Adverse Events (SUSARs).

System organ class	Preferred terms
Gastrointestinal disorders	Abdominal pain, diarrhoea, dyspepsia, gastritis, esophagitis, enterocolitis, nausea, vomiting
General disorders	Asthenia, chills, fatigue, influenzal-like illness, injection-site reactions, pyrexia
Musculoskeletal and Connective Tissue Disorders	Arthritis/arthralgia, musculoskeletal pain/weakness
Neoplasms (Benign, Malignant, and Unspecified)	Tumor pain
Skin and Subcutaneous Tissue Disorders	Pruritus, rash/desquamation, urticaria, vitiligo
Injury, poisoning and procedural complications	Radiationdermatitis

Table 5. Expected adverse events (including SAEs)

5.5.4 Deaths

No death related to the study drug ("autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine") was observed.

5.5.5 Other identified or potential safety issues

The medicinal product "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" can be administered associated with other drugs or treatments that might change its safety profile. In particular, the effects of radiotherapy within 30 days since vaccination starting or after discontinuation have been evaluated in 82 evaluable patients with advanced melanoma treated with monocyte-derived mature dendritic cells loaded with autologous tumor lysate or homogenate either enrolled in the phase I/II clinical study or under nominal request of the physician according to art. 2 comma 1 lettera f of DM 5 dicembre 2006 ("Utilizzazione di medicinali per terapia genica e per terapia cellulare somatica al di fuori di sperimentazioni cliniche e norme transitorie per la produzione di detti medicinali") and in 24 patients enrolled in an ongoing trial at our Institute (ABSIDE, EudraCT 2012-001410-41).

Sixteen out of the 82 evaluated patients underwent radiotherapy during treatment: in 7 out of the 16 seven patients the concomitant treatment was well tolerated, with no AE observed. In the other 9 patients, toxicity observed was almost exclusively related to local effects of radiotherapy (table 6) without adding additional toxicity to that expected.

Nine patients belonged to the radiotherapy arms of the ABSIDE trial and were treated with 8-12 Gy/die for three days in a metastatic lesion during vaccination and did not develop any additional adverse events.

Irradiated site	RT Dose	AE
Bone	20 Gy	No
Maxillaryregion	Notavailable	Xerostomy G1 Mucositis G2 Fatigue G1
Bone	30 Gy	No
Mediastin	25 Gy	Esofagitis G3
Pelviclymphnode	37,5 Gy	No
Retroauricularregion	40 Gy	No
Bone	Notavailable	No
Brain	Gamma-knife RT	No
Brain	Gamma-knife RT	No
GroinLymphnode (ABSIDE)	24 Gy (3 fractions)	No

NeckLymphnode (ABSIDE)	24 Gy (3 fractions)	No
Lung (ABSIDE) (3 PTS)	24 Gy (3 fractions)	No
Suprarenal gland (ABSIDE)	24 Gy (3 fractions)	No
Skin (ABSIDE)	24 Gy (3 fractions)	No
Liver (ABSIDE)	24 Gy (3 fractions)	No

Table 6. Adverse Events observed in patients concomitantly treated with radiotherapy and DC vaccine.

The 90 patients treated with vaccination (82+8) in our Institute underwent also 5 day of subcutaneous low dose Il-2 (3MUI/die), among these patients we never observed toxities other than one expected by sc IL-2 (flu like syndrom duringe the 5 days). Vaccination did not add adverse events.

Safety data of the association between DC vaccination and temozolomide (TMZ) derive also from the study by Ridolfi L. et al.[40] in which patients were treated (only for the first 6 cycles) pre-vaccination with low-dose TMZ: 75 mg/m2/day (max 100 mg/day) for 14 days. In this study were enrolled 17 deeply pre-treated advanced melanoma patients. The first 12 evaluable patients received a median TMZ dose/cycle of 1400 mg, while the remaining 5 were administered 700 mg. Only 2 patients were forced to reduce TMZ dose because of intolerance. No major toxicities were observed. The most frequent adverse events to TMZ were grade II nausea and vomiting. There were no cases of hematological toxicity. Toxicities linked to vaccination plus IL-2 were mainly flu-like syndromes (grade I-II asthenia and fever) after IL-2 administration and local reactions in the vaccine injection sites.

6. SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

The "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" described in this Investigator Brochure is an Advanced Therapy Medicinal Product for autologous use only which consists of dendritic cells obtained by in vitro differentiation of peripheral blood monocytes, isolated by leukapheresis from each patient, with IL-4 and GM-CSF. Immature DC such obtained are then loaded with an homogenate or lysate of tumor tissue obtained from the same patient, matured with a cytokine cocktail containing IL1β, PGE2, IL6, and TNF-α ("maturation cocktail"), and then intradermally administered. Its activity is exerted by inducing tumor-specific immune responses which can mediate tumor regression. Results from clinical experience in 82 patients treated either in a phase I/II clinical study or under nominal request with autologous DC loaded with autologous tumor lysate/homogenate and matured with a cytokine cocktail in subjects with advanced melanoma showed a clinical benefit (PR + SD) in 49.3% of evaluable patients.16

In 66.6% of patients treatment with the DC vaccine efficiently induced antitumor immune responses (as assessed by DTH testings). Of note, these patients experienced a significantly better overall survival than non-immunoresponsive ones (22.76vs 8.06 months in DTH+ and DTH- patients, respectively; log-rank test, p=0.0036).

6.1 Reference safety information for assessment of expectedness of serious adverse reactions

This section provides expected serious adverse reactions for regulatory reporting purposes. The information within this section does not present a comprehensive overview of the safety profile of the investigational medicinal product. (See section 5.5 for overview of the safety profile).

The "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" showed a very favorable toxicity profile: treatment-emergent adverse events (AEs) were reported for 46.34% (38/82) of all patients treated with the medicinal product "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" herein described on 24 patients enrolled in the ABSIDE study and on 10 patients enrolled in the COREVAX study. Almost all observed AE were G1-G2 adverse events related to IL-2 administration, given after each vaccine dose as immunological adjuvant. In 18 out of the 90 patients, self-limiting mild erythema, induration or pruritus at vaccine injection site was observed.

Table 7 includes Serious Adverse Reactions for the "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" considered expected for safety reporting purposes. Life-threatening and fatal adverse reactions will always be considered unexpected. Categories are based on safety reports on 90 patients treated with the "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine".

Table. 7 Reference Safety Informations. Adverse Vaccine Reactions considered expected for safety reporting purposes

Number of Subjects exposed (N): 90		
SOC	**SARs**	**N (%)**
General disorders and administration site conditions	mild erythema at vaccine injection site	**2 (2.2)**
	localized edema	**7 (7.7)**

6.2 Warnings

The medicinal product "autologous dendritic cell loaded with autologous tumor homogenate or lysate vaccine" is for exclusive AUTOLOGOUS use in a hospital setting.

It must be administered within 60' (maintained at room temperature)or 8 hours (maintained at 2-8°C) after release by the IRCCS IRST Laboratory of Somatic Cell Therapy.

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Figure 4. Flow chart of product manufacturing.