INVESTIGATIONAL MEDICINAL PRODUCT DOSSIER

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

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1. INTRODUCTION

The IMP described in this revision of the document is proposed for both monocenter and multicenter clinical trials, according to the Clinical Protocol presented to the Competent Autorithies.

Previously the IMP was intended for monocenter clinical trials only, performed in the same site as the GMP manufacturing facility is located.

In order to allow the performance of multicentric clinical trials, some additional studies regarding the stability of the starting materials and of the drug product have been performed.

The candidate clinical centers will be evaluated considering the distance from the GMP manufacturing site, in order to allow the fulfilment of the new shelf life defined.

1.1 SCIENTIFIC RATIONALE OF THE STUDY

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 1998.[4]

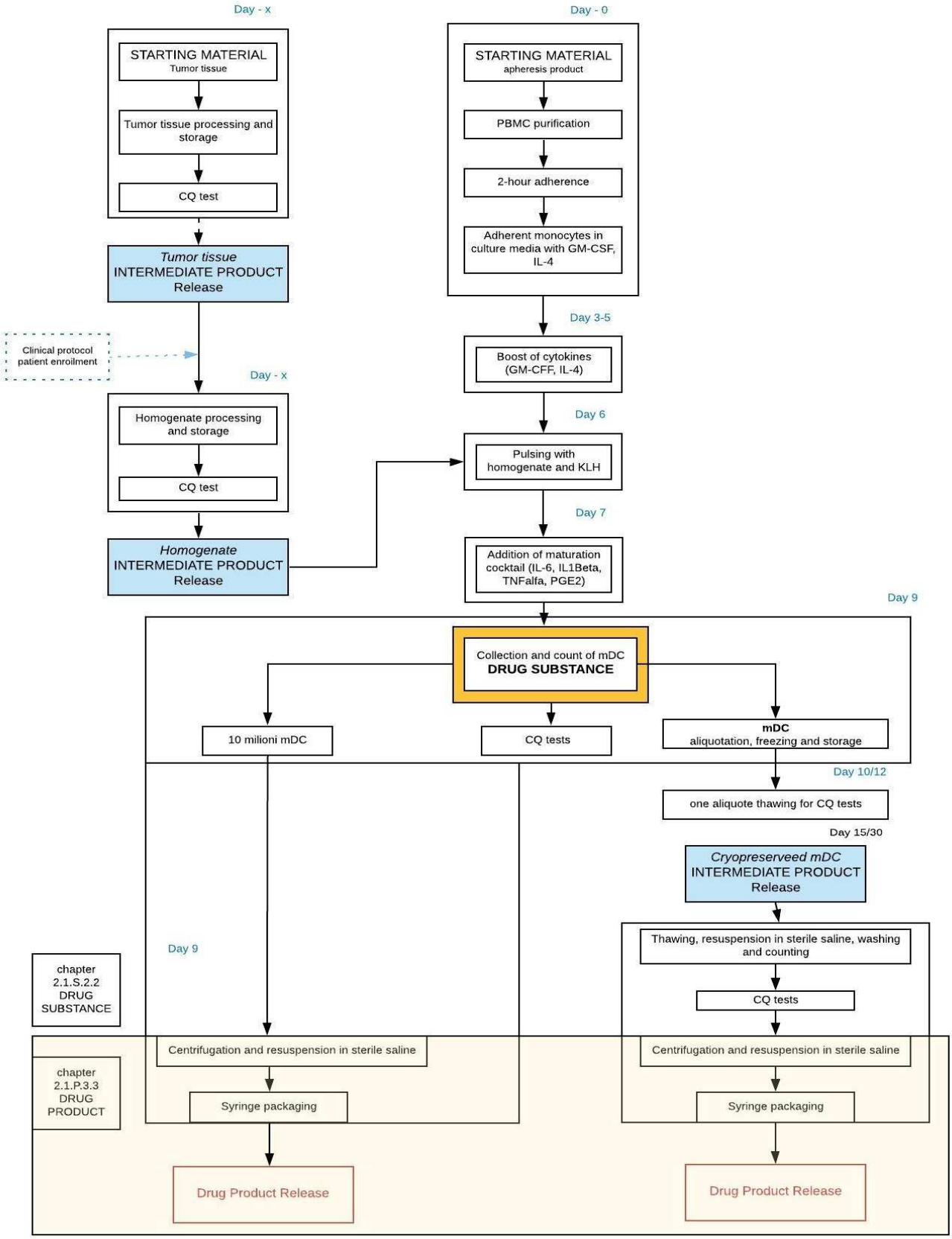
In this respect, dendritic cells (DC) loaded with tumor antigens, differentiated and matured *in vitro* have been largely shown to efficiently induce and/or potentiate tumor-specific immune responses which underlay their anticancer activity.[5,6]

The aim to use an autologous DC vaccine is to induce a specific antitumor immune response, to generate a long-term memory, withouth inducing significative side effects.

The Advanced Therapy Medicinal Product produced at IRST Cell factory is an 'Autologous dendritic cell vaccine loaded with autologous tumor homogenate', which has been utilized in 90 patients affected by metastatic melanoma in several clinical studies and in a compassionate use program, showing a very favorable safety profile and clinical activity (see section 2.1.P.2 Pharmaceutical Development of this IMPD, and sections 5.4 Clinical efficacy and 5.5 Clinical Safety of the Investigator Brochure that is an integral part of this IMPD).

The "Autologous dendritic cell vaccine loaded with autologous tumor homogenate" is produced according to the manufacturing process showed in figure 1

Fig.1: Flow chart of product manufacturing process



2.1 CHEMICAL PHARMACEUTICAL AND BIOLOGICAL DATA

2.1.S DRUG SUBSTANCE

The drug substance is composed of alive autologous mature dendritic cells pulsed with autologous tumor homogenate, resuspended in sterile saline.

2.1.S.1 GENERAL INFORMATION

2.1.S.1.1 NOMENCLATURE

Autologous dendritic cell vaccine loaded with autologous tumor homogenate.

It is an Advanced Therapy Medicinal Product as defined in DL216/2006:

1.3.1.3 'Prodotti medicinali medicinali biologici – prodotti per terapia cellulare'

1.1.1.4 'Prodotto sterile - preparato in asepsi – liquido di piccolo volume'

2.1.S.1.2 STRUCTURE

The DC used are derived from pheripheral blood monocytes (myeloid lineage).

DC size is about 10 - 15 µm

They contain abundant intracellular structures relating to antigen processing including endosomes and lysosomes.

The key morphological characteristic of mature DC is the presence of numerous membrane processes that extend out from the main cell body (similar to dendrites on neurons).

2.1.S.1.3 GENERAL PROPERTIES

DC are the most important family of professional Antigen Presenting Cells.

A main feature of DC is their phenotypic and functional plasticity: in the absence of any inflammatory or pathogenic element, most DC found in peripheral tissues and lymphoid organs have a resting, immature phenotype characterized by high endocytic capacity and low surface expression of MHC molecules and costimulatory molecules such as CD86 and CD40. However, upon interaction with microbial ligands, including TLR ligands (e.g., LPS, CpG-DNA), or upon ligation of CD40, or under influence of proinflammatory cytokines, DC rapidly acquire an activated phenotype. These mature DC have a decreased MHC class II Ag-processing capacity, an increase in filamentous actin number, the appearance of veils, an increased expression of costimulatory molecules (CD80, CD86) and of differentiation state (CD83) on the cell surface. Mature DC show a very efficient migration properties and T cell-priming ability.

2.1.S.2 MANUFACTURE

2.1.S.2.1 MANUFACTURER

The cell therapy medicinal product described in this IMPD is manufactured in the following facility only:

Immuno-Gene Therapy Factory (IGTF)

c/o IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" - IRST Via Maroncelli, 40

47014 Meldola (FC)

Tel 0543 739231 / 739222

Figure 2 shows the GMP area, the Cell Factory in gray, the Quality Control Laboratory (QC Area) in yellow, the freezing room in light blue.

Figure 3 shows the flow of GMP staff (blue arrows, for staff entrance and red arrows, for staff exit).

Figure 4 shows the flow for the introduction of material.

Figure 5 shows the flow for elimination/exit of material.

The Cell Factory has been authorized for the production of cellular therapy products (AIFA Authorization n° aM-55/2012, 27/04/2012), and visited for GMP general revisions.

It is a node of the Emilia Romagna Cell factories network (Deliberation of Giunta della Regione Emilia Romagna n°311, 23/03/2009).

The qualified person is:

Dr. Massimiliano Petrini

E-mail: massimiliano.petrini@irst.emr.it

The quality control tests of bioburden, sterility, growth promotion, endotoxin, mycoplasma are performed according to EuPharmacopoeia (See Table 6) at the GMP external laboratory Eurofins Biolab, Via Bruno Buozzi 2, Vimodrone (MI).

Fig. 2: GMP Area

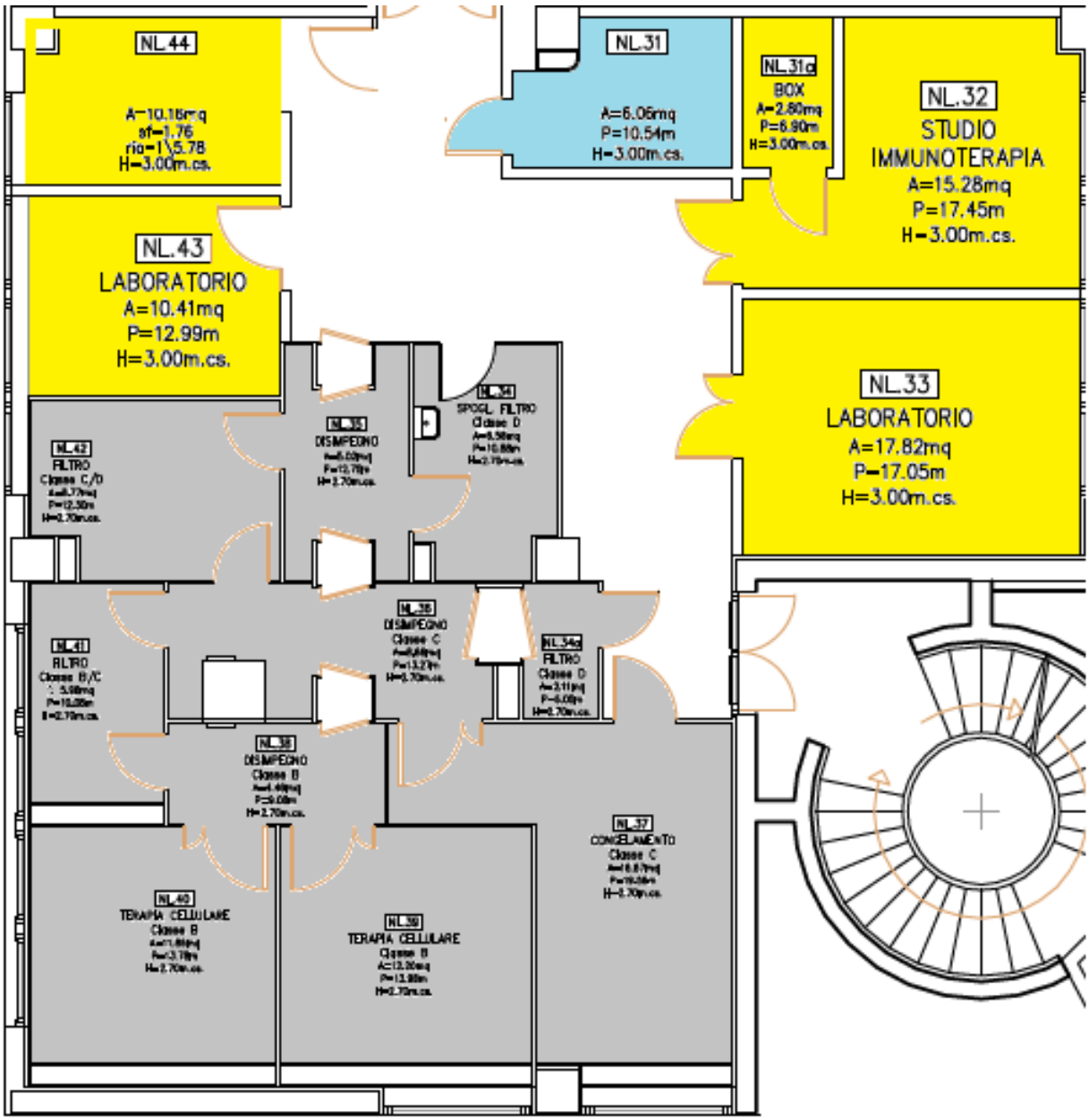
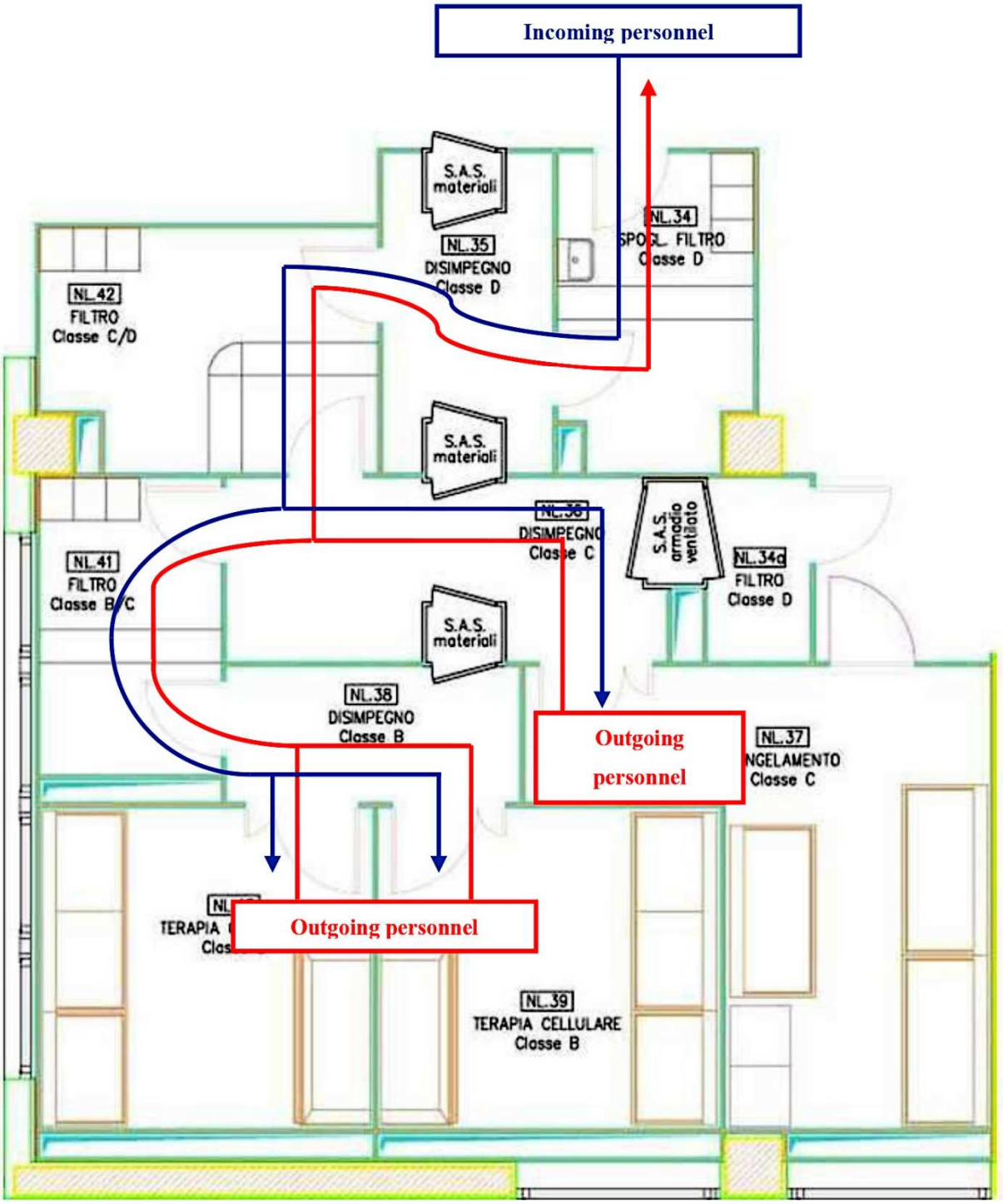


Fig. 3: Flow of GMP staff



Simultaneous entry and exit of personnel is avoided by following entry instructions

Fig. 4: Incoming material flow

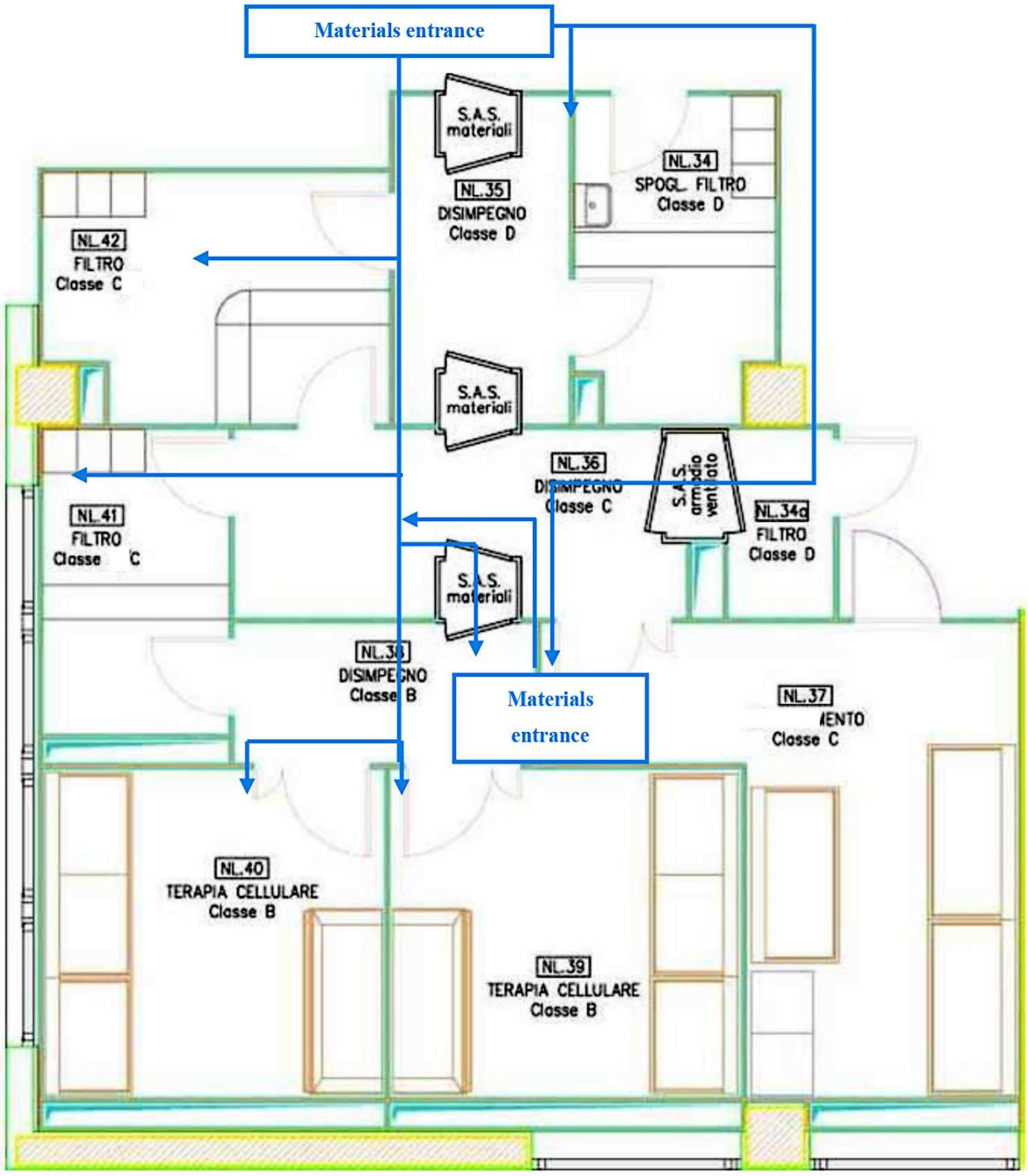
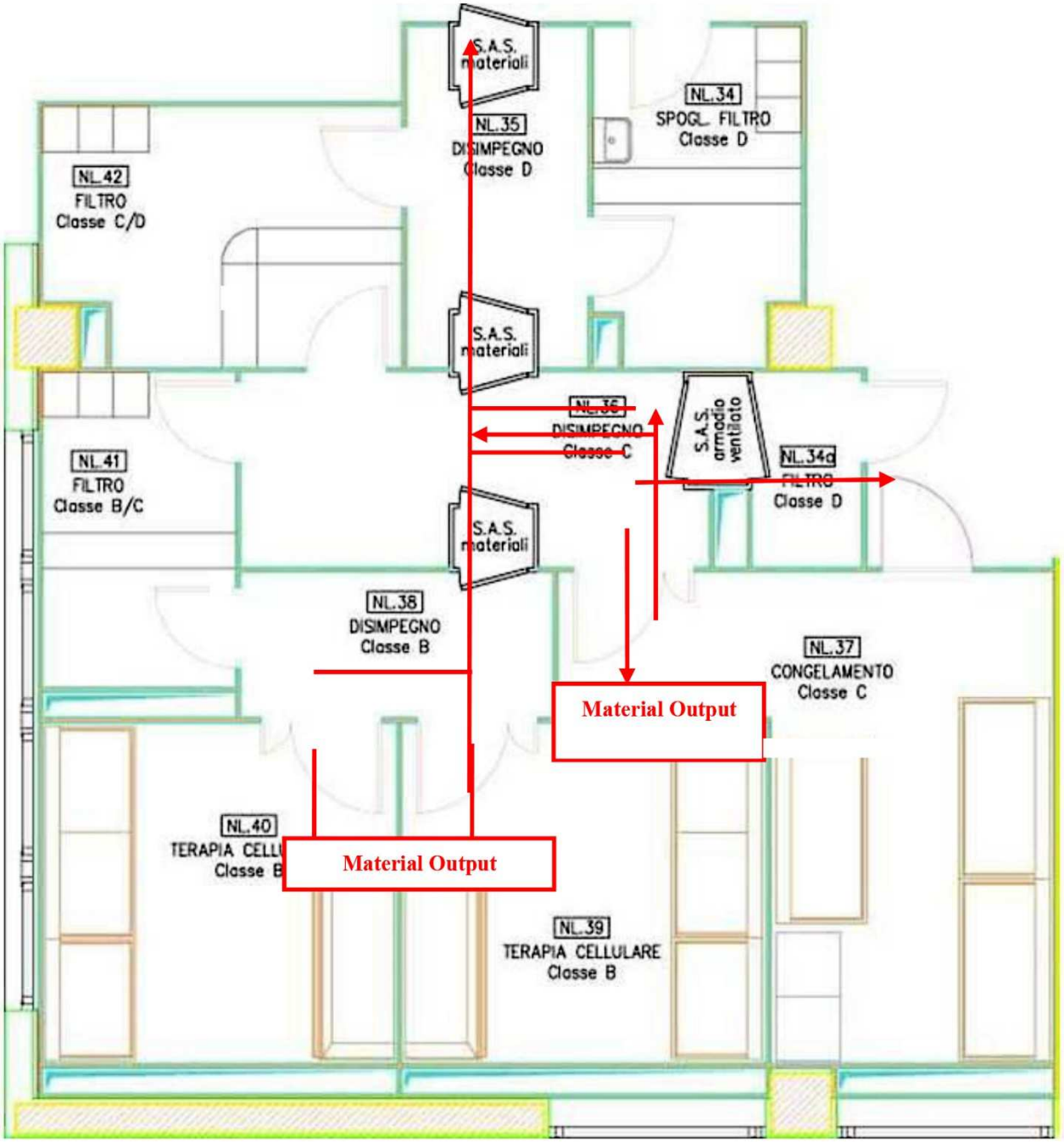


Fig. 5: Outgoing material flow



The upper passboxes (SAS materials) are used for the incoming material, while the lower passboxes are used for the outgoing material.

2.1.S.2.2 DESCRIPTION OF MANUFACTURING PROCESS AND PROCESS

The main steps to produce the drug substance are summarized in figure 1.

The drug substance is prepared from two different starting materials:

a. tumour tissue
b. apheresis product

obtained from patients tested according to DL n.16, 25/01/2010 and processed under GMP conditions.

a. The tumour tissue surgically removed from the patient, patient was divided in two representative portions. One small portion was devoted to histophatological analysis, the other was used for the production of protein homogenate. Then the tumor tissue is washed, isolated, mechanically disgregated in 0.5-1 cm^3 portions and stored at -80°C.

   When the histological diagnosis is available and confirms the presence of tumor cells and the patient is enrolled into the clinical study, the GMP manufacturing facility prepare the tumor homogenate starting from the released frozen tumour tissue, following a standardized protocol. Briefly, frozen tumor tissue was minced, mechanically disgregated using scalpel and scissors and transferred to Tube type M (Miltenyi) containing sterile NaCl 0.9% solution and mechanically dispersed again with GentleMACS™ Dissociator (Miltenyi) using installed software "RNA_02". Pellet was resuspended in sterile NaCl 0.9% solution and devoted to quality controls. Suspension was centrifuged and percolate through three filters (0.45µm, 0.22µm) and through a sterile disposable vacuum filters unit (0.22 µm) and stored in aliquots at -80 °C until use for immature dendritic cell pulsing. Protein content is determined by Comassie Bradford Protein Assay kit following manufacturer instructions.

b. When the tumor homogenate is released, the patient undergoes a leukapheresis.

The apheresis product is purified by a gradient density separation to obtain PBMC (Peripheral Blood Mononuclear Cells) which are put in culture.

The culture consists in numerous different steps:

1. PBMC undergo 2-hour adherence in plastic flasks to isolate adherent monocytes.
2. Monocytes are incubated for 6 days at 37 °C and 5% CO_2 in a culture medium supplemented with IL-4 and GM-CSF at the concentration of 1000 UI/ml, with an additional boost of cytokines at day 3 or day 5 (500 UI/ml). This step lets the monocytes differentiate into immature dendritic cells (iDC).

3. On day 6 iDC are pulsed for 16-20 hours to induce uploading of tumour antigens with:
   a. tumour homogenate (prepared as previously described) at the dose of 100µg/ml of culture (90-95% of culture volume);
   b. keyhole limpet haemocyanin (KLH) at the dose of 50µg/ml of culture (5-10% of culture volume).
4. On day 7 pulsed iDC are matured for 48 hours with a standard cocktail of cytokines: IL-6 (2000 UI/ml), TNFα (20 ng/ml), IL-1β (20 ng/ml), PGE_2 (1 µg/ml).

   On day 8 a sample of culture medium from each flask was collected for Gram staining
5. At day 9 pulsed mature dendritic cells (mDC) are collected andwashed in 0.9% sterile saline. Vitality and purity of the mature DC population is checked by Trypan Blue staining and count of both alive and dead cells.
6. The cells are finally divided:
   a. a part of cells is used to characterize the drug substance and perform the CQ test, utilized for the release of the drug product
   b. $10x10^6$ mDC are used to prepare the final product if required by the clinical protocol;
   c. the remaining mDC are frozen in autologous plasma supplemented with 10% DMSO using a controlled-rate freezing method (**intermediate product**), and stored in nitrogen liquid vapours ($13\text{-}15\times10^6$ cells/vial) (**Cryopreserved mDC**).

      This intermediate product is used to prepare the final product through additional process steps: cryopreserved mDC are thawed, resuspended in sterile NaCl 0,9% solution and counted in a Neubauer chamber using the vital stain trypan blue. mDC suspension was centrifugated and the pellet resuspended in sterile NaCl 0.9% solution at a concentration of 5×10^6 cells/ml and splitted into two syringes (10×10^6 cells) for immediate administration to the patient.

2.1.S.2.3 CONTROL OF MATERIALS

The following starting materials are utilized for the preparation of the drug substance:

Autologous tumor tissue

Autologous apheresis product

Autologous tumor tissue is provided by the Surgery Unit of the centers involved in the clinical trials according to specific procedures which regulate tissue manipulation and transportation to the IGTF IRCCS IRST.

Apheretic product is provided by Transfusional Unit of the centers involved in the clinical trial, according to specific procedures which regulate manipulation and transportation of the apheretic product to the IGTF, IRCCS IRST.

As prescribed by UE Directive 2006/17/CE and Italian Law DL 25/01/2010 n.16, after an anamnestic evaluation, patients must perform the prescribed tests within 30 days before surgical removal of tumor tissue and apheresis.

The minimum prescription for biological tests to be performed is:

- Anti-HIV-1,2;
- HbsAg (Australia Antigen) and Anti-HBc (Anticore antibodies);
- Anti-HCV Ab;
- Control algorithm for Treponema.
- IgM anti Mycoplasma pneumonia (only before surgical removal)

Negative testing for all parameters evaluated with the exception of mycoplasma is mandatory for patients' material processing in the IGTF, IRCCS IRST.

If a donor is positive to anti Mycoplasma pneumoniae IgM, the presence of mycoplasmas in the homogenate intermediate product is checked. Specifics for acceptance and approval for the starting materials are listed in table 1-2.

Test	Specific	Method
Identity	Correspondence and correct sample labeling	Visual inspection
Serological tests	Negative testing, date of testing not older than 30 days, check approval by PQ/RP/RCQ	Document check
Surgery agreement	Approval by surgeons	Check date and surgeons signs
Integrity	Primary packaging integrity	Visual inspection
Informed consents	Availability of signed and dated informed consents	Document check

Table 1: Specifics for acceptance and approval of autologous **tumor tissue**.

Test	Specific	Method
Identity	Correct bag labeling correspondent to Transfusional Unit form	Visual inspection
Serological tests	Negative testing, date of testing not older than 30 days, check approval by PQ/RP/RCQ	Document check
Delivery time	Within 24 hours after the end of the collection procedure (stored at 2-8°C)	Document check
Integrity	Primary packaging integrity	Visual inspection
Macroscopic analysis	Clots absence	Visual inspection

Table 2: Specifics for acceptance and approval of **apheresis product**

Control of ancillary materials

Ancillary materials utilized in the manufacturing process are listed in table 3.

Material	Manufacturer	Qualification	Remarks
Ficoll	Biowest	CoA	
RPMI	LONZA	CoA	
Cell Gro DC	Cell Genix	CoA	Produced in GMP and without use of animal or human-derived components
GM CSF	Cell Genix	CoA	
IL4	Cell Genix	CoA	
IL6	Cell Genix	CoA	
IL1beta	Cell Genix	CoA	
TNF alfa	Cell Genix	CoA	
ProstinE_2	Pfizer	AIC n°027114014	It is a drug approved for clinical use
PGE_2 *	Cayman Chemical	CoA	Chemical synthesis product, with no use of animal or human-derived components
Immucothel (KLH)	Biosyn Arzneimittel GmBh	AIC n° RVG18971	It is a drug approved for clinical use
DMSO	Bioniche Pharma	CoA	
NaCl 0.9%	Baxter	AIC n°030942609 (100ml) AIC n°030942611 (250 ml)	It is a perfusional solution
Autologous plasma	Transfusional Unit	See Table 4	

Table 3: List of ancillary materials. *Qualification: CoA=Certificate of Analysis* *As described in the attachement 6 (Report RC19, attachment 6) the use of Cayman Chemical PGE2 is alternative to Pfizer ProstinE_2.

Autologous plasma is provided by Transfusional Unit of the centers involved in the clinical trial, according to specific procedures which regulate manipulation and transportation of the apheretic product to the IGTF, IRCCS IRST.

Specifics for acceptance and approval of autologous plasma are listed in table 4.

Test	Specific	Methods
Identity	Correct bag labeling correspondent to Transfusional Unit form	Visual inspection
Serological tests	Negative testing, date of testing not older than 30 days, check approval by PQ/RP/RCQ	Document check
Delivery time	Within 24 hours after the end of the collection procedure (stored at 2-10°C)	Document check
Integrity	Primary packaging integrity	Visual inspection
Macroscopic analysis	Clots absence	Visual inspection

Table 4: Specifics for acceptance and approval of **autologous plasma**

Control of excipients for administration

Dendritic cells are resuspended in NaCl 0.9%
(see specifics in table 3).

2.1.S.2.4 CONTROL OF CRITICAL STEPS AND INTERMEDIATES

In process controls

All culture flasks are visually and microscopically inspected at every production step (day 0, 3 or 5, 6, 7, 8, 9) with the aim to identify possible microbiological contamination and allow to isolate or eliminate contaminated flasks.

The process of freezing of mDC in aliquots (**intermediate products**) must initiate in short time (within 20 minutes since their preparation). Another critical point is the adherence of the real freezing curve to the optimal programmed curve, evaluated throught the following parameters:

- $T_{critical}$ of the sample (i.e., freezing point of cells) ≥ -12°C
- Freezing velocity of the sample until -20°C of 1°C/min ± 0.5°C/min

According to EU-cGMP Vol.4, production areas and operators are controlled during production activities by microbiological and particulate air monitoring.

Control of intermediate products

The following intermediate products are utilized for the preparation of "Autologous dendritic cell loaded with autologous tumor homogenate":

Tumor tissue

Homogenate

Cryopreserved mDC

All the intermediates must be checked according to the in process controls and release criteria listed in table 5,6,7.

Final release criteria

Test on Tumor tissue	Limits	Method
Histologic Diagnosis	Positive	Pathologist report

Table 5: Criteria for final release of intermediate product **Tumor tissue**

In process control and Final release criteria

Homogenate – In process controls		
Test on Homogenate	**Limits**	**Method**
Bioburden	$<10^7$ CFU/20ml	TAMC According to EuPh 2.6.12
Homogenate – Final release		
Test on Homogenate	**Limits**	**Method**
Endotoxin	<1748EU/mg	LAL test According to EuPh 2.6.14
Sterility	Sterile	According to EuPh 2.6.27
Growth promotion	Fertile	According to EuPh 2.6.27
Mycoplasma *	Absent	According to EuPh 2.6.7, NAT method

Table 6.: Criteria for in process controls and final release of intermediate product **Homogenate**
*** the test for the presence of mycoplasma in the homogenate is performed only if the donor is positive for IgM anti Mycoplasma pneumoniae at the time of donation of tumor tissue.**

Release criteria

Test on drug substance	Limits	Method
Sterility (Microbiological examination of cell-based preparation)	Sterile	According to EuPh 2.6.27
Endotoxin	≤ 0.5EU/ml	According to EuPh 2.6.14, kinetic method D
Mycoplasma	Absent	According to EuPh 2.6.7, NAT method
HLA-DR	> 60%	Flow cytometry
CD80	> 50%	Flow cytometry
CD86	> 60%	Flow cytometry
CD83	> 40%	Flow cytometry
Test on thawed mDC	**Limits**	**Method**
Vitality (%)	> 70%	Vital staining
Purity (%)	> 60%	Vital staining
Sterility	Sterile	According to EuPh 2.6.27

Table 7: Criteria for release of intermediate product **Cryopreserved mDC**.

2.1.S.2.5 PROCESS VALIDATION AND/OR EVALUATION

The data on process validation are presented in par. 2.1.P.3.5, because the validation concerns the entire process from starting materials to final product.

2.1.S.2.6 MANUFACTURING PROCESS DEVELOPMENT

Up to 2010 the drug substance, obtained directly from the apheresis product or from the Cryopreserved PBMC, was used to prepare the drug product without any other intermediate step.

During 2010 an additional manufacturing process step was introduced: a part of the drug substance was frozen in aliquots as intermediate product (Cryopreserved mDC) and stored in nitrogen liquid vapours. Cryopreserved mDC were utilized to prepare the drug product, following the steps described in chapter 2.1.S.2.2 and 2.1.P.3.3.

The validation data on this additional manufacturing process step are showed in chapter 2.1.P.3.5.

The entire manufacturing process was analysed and authorized during the AIFA site visits.

2.1.S.3 CHARACTERIZATION

2.1.S.3.1 ELUCIDATION OF STRUCTURE AND OTHER CHARACTERISTICS

Not applicable.

2.1.S.3.2 IMPURITIES

It is accepted that the drug substance may contain proportion not higher than 40% of cells which did not differentiate to dendritic cells. This is dependent on the selection method of monocyte, which employs plastic adherence of PBMC. This method do not allow to efficiently select monocytes from PBMC and carry-over contaminant cells during the procedure. A systematic characterization of contaminant cells have not been performed, although in a limited number of cases FACS analysis allowed to identify B and T cell, macrophage, and granulocytes as contaminants.

However, cellular impurities found in the final product, did not seem to influence the toxicity profile of the product which is overall low.

2.1.S.4 CONTROL OF DRUG SUBSTANCE

2.1.S.4.1 SPECIFICATION

The drug substance is evaluated for the following characteristics:

Test		Limits	Method
Vitality (%)		> 70%	Vital staining According to EuPh 2.7.29
Purity (%)		> 60%	Vital staining According to EuPh 2.7.29
Direct bacterial examination		Negative	Gram staining
Sterility (Microbiological examination of cell-based preparation)		Sterile	According to EuPh 2.6.27
Endotoxin		≤ 0.5EU/ml	According to EuPh 2.6.14, kinetic method D
Mycoplasma		Absent	According to EuPh 2.6.7, NAT method
Flow cytometric Phenotyping	HLA-DR	> 60%	Flow cytometry
	CD80	> 50%	
	CD86	> 60%	
	CD83	> 40%	

Table 8: Quality controls of **Drug substance**

2.1.S.4.2 ANALYTICAL PROCEDURES

The analytical procedures are listed in Table 8.

2.1.S.4.3 VALIDATION OF ANALYTICAL PROCEDURES

All tests, excepting direct bacterial examination and flow cytometric phenotyping, have been validated. All the methods are standardized and are adequate to detect significant deviations from the specifications.

2.1.S.4.4 BATCH ANALYSES

Not applicable

2.1.S.4.5 JUSTIFICATION OF SPECIFICATIONS

Test		Limits	Justification
Vitality (%)		> 70%	Historic and literature data
Purity (%)		> 60%	Historic and literature data
Direct bacterial examination		Negative	Sterile biologic product, non sterilizable therefore the absence of microbiological contamination is required
Sterility (Microbiological examination of cell-based preparation)		Sterile	Sterile biologic product, non sterilizable; therefore, the absence of microbiological contamination is required
Endotoxin		≤ 0.5EU/ml	The limit was calculated considering the most critical conditions for risk assessment (intratecal administration for 70Kg of weight at a speed of 20 ml/h). The limit was calculated at 0,7 EU/ml, and was lowered at 0.5 EU/ml in order to provide increased security.
Mycoplasma		Absent	Sterile biologic product, non sterilizable; therefore, the absence of microbiological contamination is required
Flow cytometric Phenotyping	HLA-DR	> 60%	Historic and literature data
	CD80	> 50%	
	CD86	> 60%	
	CD83	> 40%	

Table 9: Justification of specifications

The drug substance is evaluated for vitality and purity in order to prepare a drug product consisting in alive cells with a representative percentage of autologous mature DC pulsed with autologous tumor homogenate.

The Gram staining is performed to assess the absence of bacterial contaminants before preparing the drug product, because the sterility test according to Eu-Ph 2.6.27 takes one week to produce result.

The phenotype caracterization includes HLA-DR, CD80, CD86, CD83 to assess mature dendritic cells identity. HLA-DR is involved in the mechanism of antigen presentation. CD80 and CD86 are costimulatory molecules necessary for T cell interaction and activation and are markers of APC. CD83 is a specific marker of mDC, whose expression is higher after tumor antigens loading and after maturation.

2.1.S.5 REFERENCE STANDARDS OR MATERIALS

Not applicable.

2.1.S.6 CONTAINER CLOSURE SYSTEM

Autologous pulsed mature dendritic cells resuspended in sterile saline are contained in a 50ml sterile tube with screw cap. This container is filled and closed in Class A area.

2.1.S.7 STABILITY, STORAGE CONDITIONS, TRANSPORT AND LOGGING

The drug substance is immediately used to prepare the drug product.

2.1.P DRUG PRODUCT

The main steps to produce the drug product are summarized in figure 1.

2.1.P.1 DESCRIPTION AND COMPOSITION OF THE DRUG PRODUCT

The drug product is a suspension of autologous 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 5×10^6 cells/ml, packed in 2 insulin syringes, ready for use.

2.1.P.2 PHARMACEUTICAL DEVELOPMENT

The product described in this IMPD is produced by the Immuno-Gene Therapy Factory (IGTF), IRCCS IRST since 2001 (Clinical trial approved by Istituto Superiore di Sanità,
N°800/II A.48.3/2099 "Vaccinazione con cellule dendritiche in pazienti affetti da melanoma").
After completion of the phase I/II clinical trial in 2009 (data reported in the IB), the vaccine has been produced and utilized in the clinic according to the DM 21 dicembre 2007 ("Disposizioni in materia di autorizzazione alla produzione di medicinali"), under nominal request of the physician (art.5 D.L. 219/06) and classified as "Prodotto Consolidato" (autocertification sent to the Ministry of Health on 29 July 2008, 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"; referente Dr. Ruggero Ridolfi).
Until 2009, the product has been produced in a dedicated laboratory, according to quality assurance procedures.
Starting from late 2009, a few lots of the product had been produced in the IRST Cell Factory according to GMP procedures. From 2010 all lots have been manufactured under GMP conditions and process validations have been performed.[7-9]
On april 2012 the Cell Factory has been authorized for the production of cellular therapy products (AIFA Authorization n° aM-55/2012, 27/04/2012 and visited for GMP general revisions.
From late 2013 all patients have been enrolled in clinical study protocols.

2.1.P.2.1 COMPONENTS OF THE MEDICINAL PRODUCT

No additional relevant informations are provided.

2.1.P.2.2 MEDICINAL PRODUCT

No additional relevant informations are provided.

2.1.P.2.3 MANUFACTURING PROCESS DEVELOPMENT

See chapter 2.1.S.2.6. There are no additional informations about the development of final production steps from the drug substance to the drug product.

2.1.P.2.4 CONTAINER CLOSURE SYSTEM

No development informations are provided.

The drug product is packaged in two ready for use sterile insuline syringes to facilitate the intradermal administration to the patient, that must be performed within 8 hours after the drug product is prepared.

2.1.P.2.5 MICROBIOLOGICAL ATTRIBUTES

Not applicable

2.1.P.2.6 COMPATIBILITY

No relevant informations are available.

2.1.P.3 MANUFACTURE

2.1.P.3.1 MANUFACTURER

The cell therapy medicinal product described in this IMPD is manufactured in the following facility only:

Immuno-Gene Therapy Factory (IGTF)
c/o IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" – IRST S.r.l.
Via Maroncelli, 40/42 - 47014 Meldola (FC)
Tel 0543 739231 / 739222

2.1.P.3.2 BATCH FORMULA

Not applicable

2.1.P.3.3 DESCRIPTION OF MANUFACTURING PROCESS AND PROCESS CONTROLS

The drug product can be produced:

1. starting from the drug substance obtained directly from the apheresis product the drug substance is centrifuged at 1000rpm for ten minutes. The supernatant is eliminated. The pellet (autologous mature dendritic cells pulsed with autologous tumor homogenate) is resuspended in 2ml of 0.9% sterile saline and transferred in 2 ready for use sterile insuline syringe (See figure 6).

2. starting from the intermediate product Cryopreserved mDC (chapter 2.1.S.2.2). After thawing, resuspending with sterile saline and counting, pulsed mDC are centrifuged at 1000rpm for ten minutes. The supernatant is eliminated. The pellet (autologous mature dendritic cells pulsed with autologous tumor homogenate) is resuspended in 2ml of 0.9% sterile saline and transferred in 2 ready for use sterile insuline syringe (See figure 6).

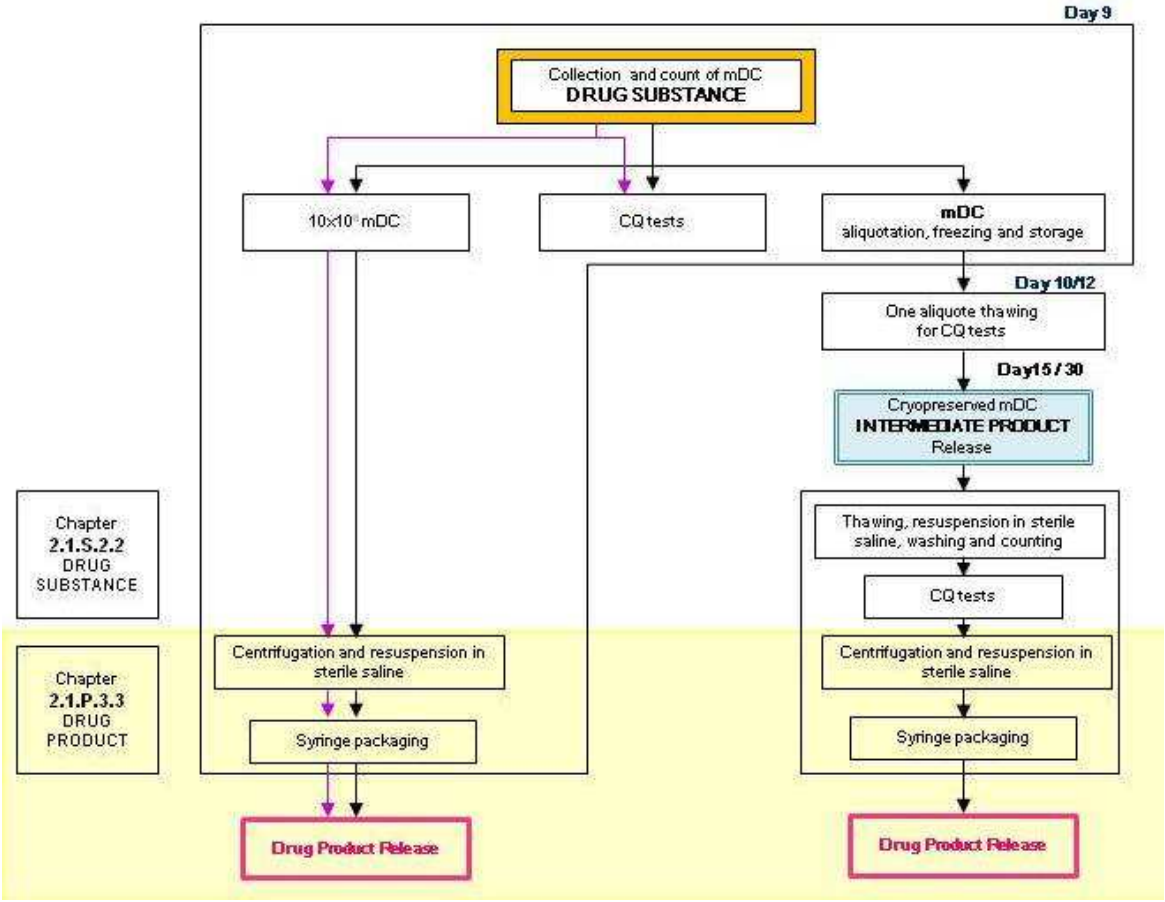


Figure 6: Final steps of drug product preparation

2.1.P.3.4 CONTROLS OF CRITICAL STEPS AND INTERMEDIATES

Not applicable

2.1.P.3.5 PROCESS VALIDATION AND/OR EVALUATION

<u>First validation</u>

In 2010 it has been performed a concomitant process validation on freshly prepared drug product for each vaccine dose. Three consecutive batches obtained from apheretic product and three consecutive batches obtained from the intermediate product Cryopreserved PBMC were evaluated. Data analysis of these 6

batches showed that the processing method systematically allows the production of a product fulfilling the release specifications.

Second validation

A prospective validation of the biological characteristics of the drug product prepared after thawing of the intermediate product "cryopreserved mDC" has been performed in 2010 on three lots obtained from three consecutive apheretic procedures (Table 10).

Fresh drug product and drug product prepared after thawing the corresponding batch of intermediate product Cryopreserved mDC (after 1 week of cryopreservation) have been analyzed for vitality, purity and phenotypic characteristics.

A functional method measuring the potency of dendritic cells have been also employed on the same samples. This method is a modification of a published validated functional assay which measure the costimulatory ability of dendritic cells,[10,11] a functional property that is accepted by EMA as a direct measure of the potency of dendritic cell vaccines.[12]

Briefly, dendritic cells are cocoltured in quadruplicate with at least three batches of allogeneic immunomagnetically isolated CD3+ T cells for 24 hours, at a DC:T cells ratio of 1:10 (105 T cells/well), in the presence of substimulating concentrations of the anti-CD3 antibody OKT3. We utilized IFN-γ ELISPOT instead of the original ^{3}H thymidine proliferation assay as a read-out system, as it allows to identify a Th1-specific costimulatory activity of dendritic cells. Results are expressed as the average spot number obtained for all reference allogeneic T cells.

In addition, process validation also evaluated the freezing step by measuring the time interval elapsed between aliquot preparation and freezing start, as well as the $T_{critical}$ of the sample during the freezing steps.

The results demonstrate that freezing, storing and thawing does not significantly alter the biological profile of the drug product.

Test	Limits	BATCHES					
		V-OP026-DC05(A2)5		V-OP028-DC06(A2)5		V-OP021-DC04(A2)9	
		Fresh mDC	Thawed mDC	Fresh mDC	Thawed mDC	Fresh mDC	Thawed mDC
Vitality (%)	> 70%	92	83	94	82	95	88
Purity (%)	> 60%	76	76	62	67	67	67
Sterility	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile
Endotoxin	<0.5EU/ml	<0.5EU/ml	Not performed	<0.5EU/ml	Not performed	<0.5EU/ml	Not performed
Micoplasma	Absent	Absent	Not performed	Absent	Not performed	Absent	Not performed
HLA-DR	> 60%	94	80	91	94	93	85
CD80	> 50%	96	92	91	97	95	94
CD86	> 60%	99	98	97	99	99	99
CD83	> 40%	91	87	92	92	90	90
Potency	> 70%	100%	149%	100%	79%	100%	122%

Table 10: Comparison of biological characteristics of drug product prepared from Cryopreserved mDC or from fresh drug substance.

Process validation monitoring

Annual Product Quality Review did not detect significant changes or negative trends, demonstrating that the validated manufacturing process is under control and allows to obtain a product fulfilling the required specifications (Table 11-12).

2.1.P.4 CONTROL OF EXCIPIENTS

2.1.P.4.1 SPECIFICATIONS

The 0.9 % sterile saline used in the formulation of the drug product is a perfusional solution with AIC n° 030942609 and n°030942611

2.1.P.4.2 ANALYTICAL PROCEDURES

Not applicable

2.1.P.4.3 VALIDATION OF ANALYTICAL PROCEDURES

Not applicable

2.1.P.4.4 JUSTIFICATIONS OF SPECIFICATIONS

Not applicable.

2.1.P.4.5 EXCIPIENTS OF HUMAN OR ANIMAL ORIGIN

No allogeneic human substances nor excipients of animal origin are employed.

2.1.P.4.6 NOVEL EXCIPIENTS

Not applicable.

2.1.P.5 CONTROL OF MEDICINAL PRODUCT

2.1.P.5.1 SPECIFICATIONS

The drug product is prepared starting from the drug substance directly produced from apheresis product (A) or the intermediate product 'Cryopreserved mDC' (B) (See Figure 1).

The release of the drug product (A) requires two steps:

1. Partial release: first authorization by the Qualified Person to deliver the product for the administration to the patient in the presence of the CQ test of vitality, purity, direct bacterial examination
2. Final release: final authorization by the Qualified Person in the presence of the CQ test of phenotipe caracterization (HLA-DR, CD80, CD86, CD83), sterility, endotoxin and mycoplasma (Table 11)

The CQ testing for the drug product release (A) are performed on the drug substance (see chapter 2.1.S.4.1), just before the drug product preparation.

PARTIAL RELEASE			
Test		**Limits**	**Method**
Vitality (%)		> 70%	Vital staining According to EuPh 2.7.29
Purity (%)		> 60%	Vital staining According to EuPh 2.7.29
Direct bacterial examination		Negative	Gram staining
FINAL RELEASE			
Test		**Limits**	**Method**
Sterility		Sterile	According to EuPh 2.6.27,
Endotoxin		≤ 0.5EU/ml	According to EuPh 2.6.14, kinetic method D
Micoplasma		Absent	According to EuPh 2.6.7, NAT method
Flow cytometric Phenotyping	HLA-DR	> 60%	Flow cytometry
	CD80	> 50%	Flow cytometry
	CD86	> 60%	Flow cytometry
	CD83	> 40%	Flow cytometry

Table 11: Criteria for partial and final release of drug product (A) or (B)

The release of the drug product (B) requires one step:

1. Final release: final authorization by the Qualified Person in the presence of the CQ test of vitality and purity.

The CQ samples for the drug product release (C) are prepared from the intermediate product Cryopreserved mDC thawed, washed in 0.9% sterile saline and counted, just before the drug product preparation. (Table 12).

FINAL RELEASE		
Test	**Limits**	**Method**
Vitality (%)	> 70%	Vital staining According to EuPh 2.7.29
Purity (%)	> 60%	Vital staining According to EuPh 2.7.29

Table 12: Criteria for final release drug product (C).

2.1.P.5.2 ANALYTICAL PROCEDURES

See chapter 2.1.S.4.2

2.1.P.5.3 VALIDATION OF ANALYTICAL PROCEDURES

See 2.1.S.4.3.

2.1.P.5.4 BATCH ANALYSES

According to EU-cGMP every year all product lots are evaluated and data are analysed in the corresponding Product Quality Review.

The results on drug product conformity to release parameters (years 2010-2011, 2012, 2013, 2014, 2015, 2016-2017-2018-2019) are summarized in Table 13.

	N° of lots	**Partial release passed**	**Final relase passed**
Product lots (2010)	n=37	97.30%	91.90%
Product lots (2011)	n=55	98.10%	92.70%
Product lots (2012)	n=53	100%	94,3%
Product lots (2013)	n= 64	100%	100%
Product lots (2014)	n= 32	100%	100%
Product lots (2015)	n= 27	100%	100%
Product lots (2016)	n= 43	100%	100%
Product lots (2017)	n=66	100%	100%
Product lots (2018)	n= 45	100%	100%
Product lots (2019)	n= 22	100%	100%

Table 13: Product quality review data (2010-11, 2012, 2013, 2014, 2015, 2016-2019)

Analysis of drug products manufactured in 2010, 2011, 2012 that did not fulfil criteria for final release are shown in table 14.

Lot number	Year	Partial release	Reason	Final release	Reason
V-OP02-DC01(A2)9	2010	NO	Purity <60%	NO	Phenotypic characterization absent
V-OP02-DC01(A2)10	2010	YES	n.a.	NO	Phenotypic characterization absent
V-OP055-DC011(A1)1	2011	NO	Not sterile	NO	Not sterile
V-OP028-DC06(A2)10	2011	YES	n.a.	NO	Phenotypic characterization absent
V-OP028-DC06(A2)11	2011	YES	n.a.	NO	Phenotypic characterization absent
V-OP063-DC012(A1)7	2011	YES	n.a.	NO	Phenotypic characterization absent
V-OP054-DC07(A1)3	2011	YES	n.a.	NO	Sterility, Mycoplasma and endotoxin determination absent
V-OP063-DC012(A2)10	2012	YES	n.a.	NO	Phenotypic characterization absent
V-OP063-DC012(A2)11	2012	YES	n.a.	NO	Phenotypic characterization absent
V-OP063-DC012(A3)15	2012	YES	n.a.	NO	Phenotypic characterization absent

Table 14: Product quality review 2010-2011-2012: analysis of non-conformities

2.1.P.5.5 CHARACTERIZATION OF IMPURITIES

It is accepted that in the final product a minor quote of mononuclear cells not differentiated to dendritic cells is present. This is dependent on the selection method of monocyte, which employ plastic adherence of PBMC. These impurities, however, did not induce adverse events neither affect activity of the product.

2.1.P.5.6 JUSTIFICATION OF SPECIFICATIONS

See 2.1.S.4.5.

2.1.P.6 REFERENCE STANDARDS

Not applicable.

2.1.P.7 CONTAINER CLOSURE SYSTEM

Autologous pulsed mature dendritic cells resuspended in 0.9% sterile saline are contained in two 1ml ready-for-use sterile insulin syringes. These containers are filled and closed in Class A area and report the product identity. The syringes are packed in their original packaging labeled and put in a plastic bag reporting the product and the protocol identity, according to EU-cGMP Annex XIII.

2.1.P.8 STABILITY

<u>Drug product stability in syringe</u>

As the formulated product is composed of live cells, we evaluated the stability of dendritic cells stored within the syringe utilized for its administration. Four batches manufactured outside and 2 batches inside the Cell Factory have been evaluated for stability.

Dendritic cells have been stored at room temperature in syringe and vitally counted after different times. Vitality, i.e., the percentage of vital cells on total cells (dead + live), and purity (the percentage of mature dendritic cells on total live cells) have been recorded and shown in table 15. Data analysis showed that the formulated product in syringe did not reach the critical time (T_c), i.e., the time required to lower cellular product vitality under 70% and purity under 60%, after 120'.

STABILITY AT ROOM TEMPERATURE		
Time	**Average product vitality (range)**	**Average product purity (range)**
0'	91 (83-96)	83 (64-90)
15'	89 (75-93)	81 (61-90)
30'	87 (80-98)	78 (61-91)
45'	87 (80-93)	84 (61-95)
60'	88 (74-96)	82 (64-94)
75'	87 (84-93)	88 (62-97)
90'	84 (71-96)	87 (61-96)
105'	85 (73-97)	85 (60-96)
120'	81 (70-88)	84 (61-98)

Table 15: Analysis of product stability. Average values and range for the 6 batches analyzed are shown at different time intervals after thawing.

Stability of drug product obtained from intermediate product Cryopreserved mDC

From 2012 to 2015 a stability programme on drug product has been performed.

The parameters evaluated were vitality, purity, phenotype characteristic (HLA-DR, CD80, CD86, CD83), sterility and potency.

The intermediate products Cryopreserved mDC of three different lots were thawed after 3, 6, 9, 12, 18, 24 months after freezing to prepare the drug product, which were compared to the corresponding drug product obtained directly from apheresis product.

The programme results are reported below and show that drug product obtained from intermediate product Cryopreserved mDC is stable until 24 months of storage.

All thawed batches were found to be sterile.

Table 16: Results of vitality and purity.

Acceptability limits: vitality > 70%

purity > 60%

Batch V-OP099-DC017(A2)	VITALITY (%)	PURITY (%)	COMPLIANT
Fresh	94	63	yes
Thawed 12a week	95	72	yes
Thawed 24a week	98	80	yes
Thawed 36a week	94	75	yes
Thawed 47a week	99	71	yes
Thawed 75a week	90	77	yes
Thawed 99a week	83	62	yes

Batch: V-OP0111c-DC019(A1)	VITALITY (%)	PURITY (%)	COMPLIANT
Fresh	95	71	yes
Thawed 11a week	95	86	yes
Thawed 22a week	90	71	yes
Thawed 33a week	96	65	yes
Thawed 46a week	96	68	yes
Thawed 73a week	90	75	yes
Thawed 107a week	93	78	yes

Batch: V-OP0115-DC021(A1)	VITALITY (%)	PURITY (%)	COMPLIANT
Fresh	98	61	yes
Thawed 12a week	88	62	yes
Thawed 25a week	92	61	yes
Thawed 42a week	92	61	yes
Thawed 57a week	91	62	yes
Thawed 81a week	95	62	yes
Thawed 102a week	87	62	yes

Table 17: Phenotype results

Acceptability limits: HLA-DR > 60%
CD80 > 50%
CD86 > 60%
CD83 > 40%

Batch: V-OP099-DC017(A2)	HLA-DR (%)	CD80 (%)	CD86 (%)	CD83 (%)	Compliant
Fresh	94	97	99	91	yes
Thawed 12a week	93	97	99	93	yes
Thawed 24a week	79	90	97	92	yes
Thawed 36a week	93	98	99	95	yes
Thawed 47a week	86	96	98	93	yes
Thawed 75a week	94	96	98	90	yes
Thawed 99a week	96	97	99	98	yes

Batch: V-OP0111c-DC019(A1)	HLA-DR (%)	CD80 (%)	CD86 (%)	CD83 (%)	Compliant
Fresh	97	99	99	71	yes
Thawed 11a week	85	88	93	67	yes
Thawed 22a week	96	96	98	69	yes
Thawed 33a week	93	97	98	68	yes
Thawed 46a week	98	99	99	70	yes
Thawed 73a week	90	95	98	66	yes
Thawed 107a week	98	97	98	67	yes

Batch: V-OP0115-DC021(A1)	HLA-DR (%)	CD80 (%)	CD86 (%)	CD83 (%)	Compliant
Fresh	85	87	97	77	yes
Thawed 12a week	91	97	99	81	yes
Thawed 25a week	93	99	99	80	yes
Thawed 42a week	80	93	95	83	yes
Thawed 57a week	83	92	97	79	yes
Thawed 81a week	89	99	99	79	yes
Thawed 102a week	93	98	99	80	yes

Potency Test:

The costimulatory activity of dendritic cells was measured using a test described in the literature and validated (Shankar G, Bader R, Lodge PA. The COSTIM bioassay: a novel potency test for dendritic cells. J Immunol Meth 2004; 285: 293-299 and Shankar G, Fourrier MS, Grevenkamp MA, Lodge PA. Validation of the COSTIM bioassay for dendritic cell potency. J Pharm Biomed Anal 2004; 36: 285-294), modified regarding the read-out mode (IFN-gamma ELISPOT vs. proliferation, replacement also foreseen in the reference work).

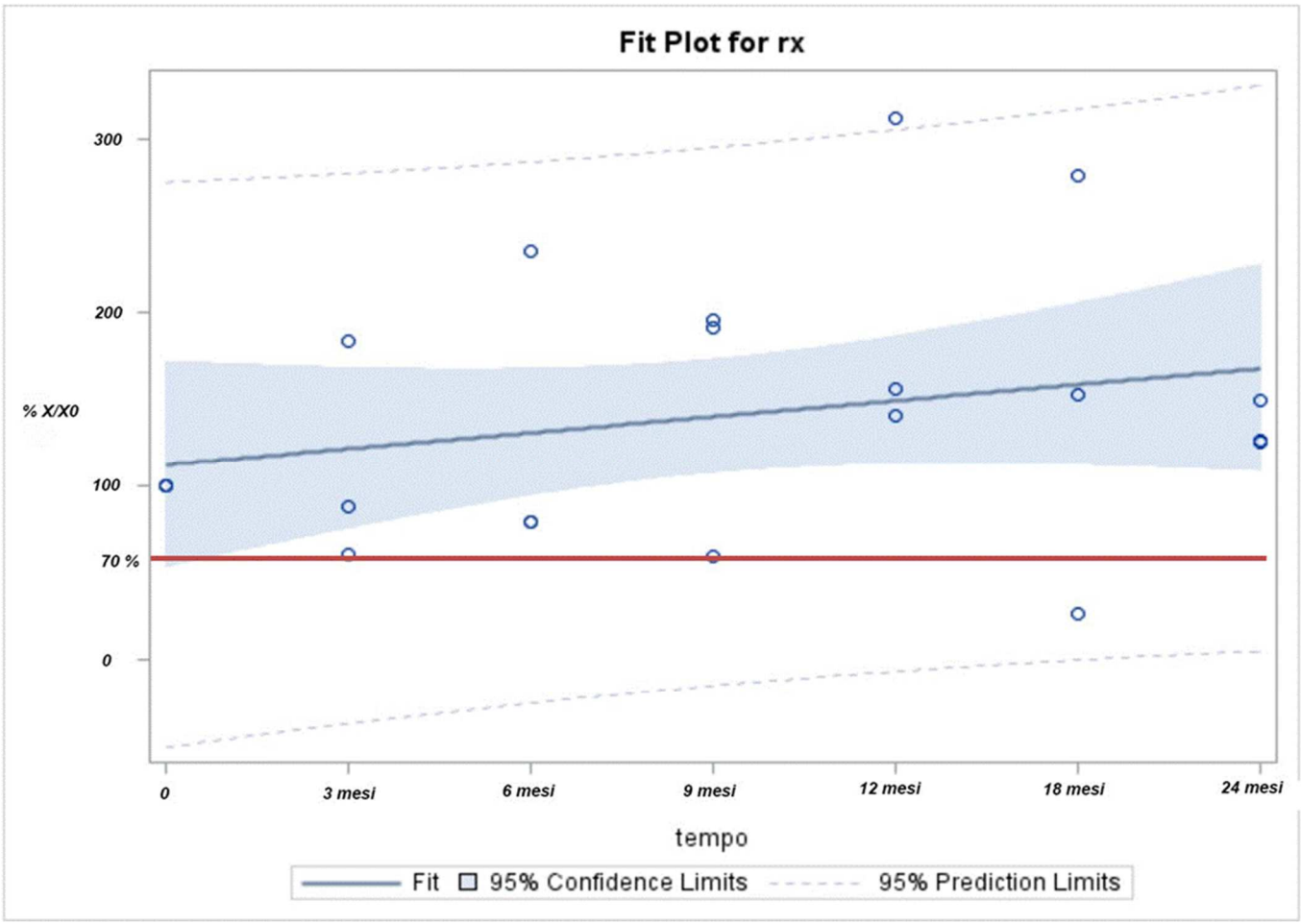
In this test, allogeneic T lymphocytes (coming from 3 healthy) were stimulated for 24 hours with fresh dendritic cells or thawed dendritic cells, at a ratio of 10:1, in the presence of substimulating concentrations (0.02 µg / ml and 4 µg / ml) and not of the antibody –CD3 OKT3.

The determinations were performed in quadruplicate and the results were analyzed by evaluating for each condition the average spot number obtained for all reference allogeneic T cells for each donor, excluding the replicates whose values were not included within the mean range +/- 1 standard deviation.

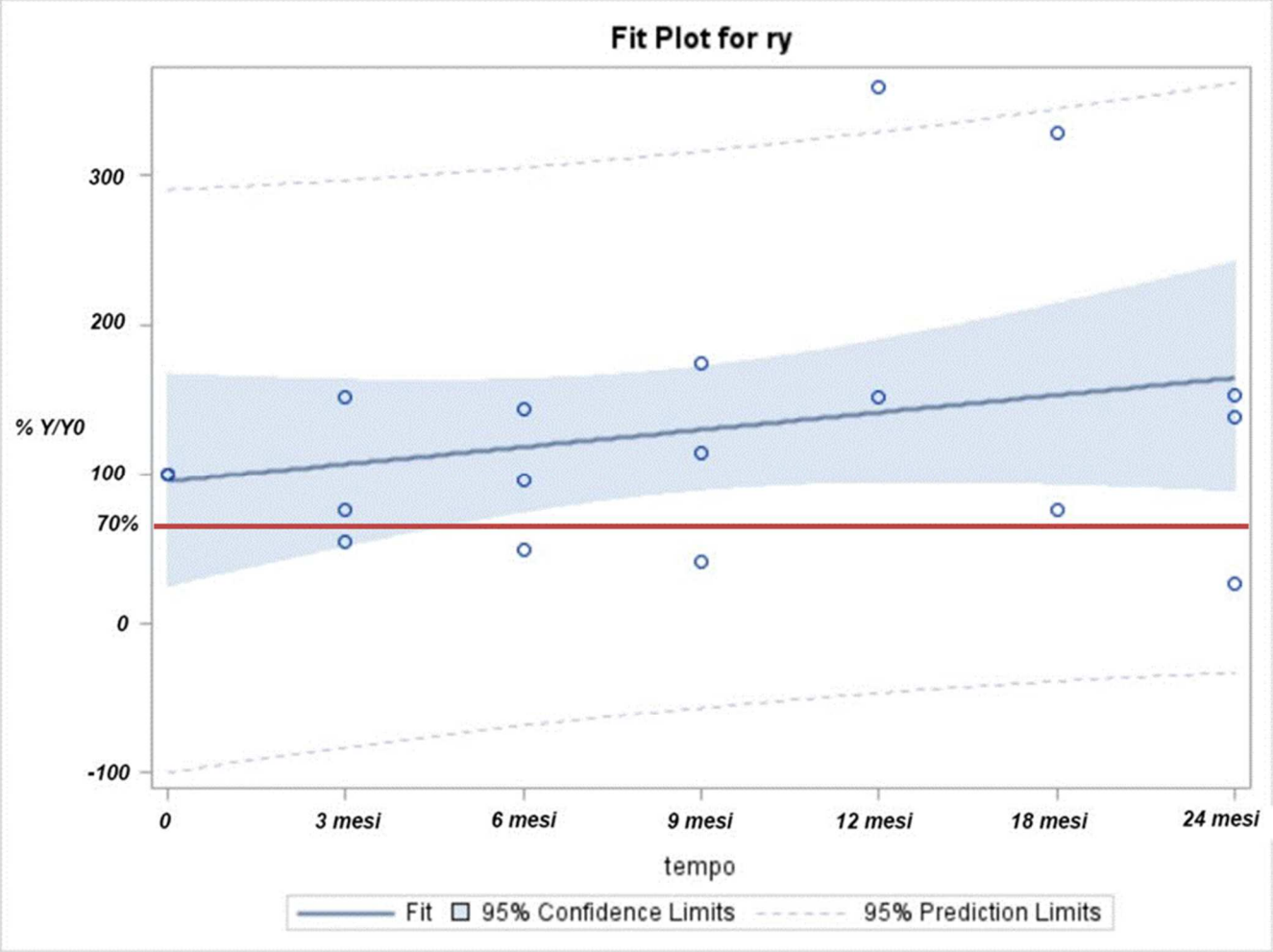
An initial evaluation of the poolability of the lots was performed and the results indicated that the stability data relating to the 3 lots did not show statistically different slopes at the significance level of 0.25 (as indicated in section B.2.2.1 of the guideline). This result then directed the subsequent analysis towards performing the regression analysis on the overall data relating to the 3 lots. As can be seen in graphs

below, the lower limit of the 95% confidence interval of the regression line is higher than the acceptance limit of 70% of the fresh product activity up to 24 months.

Graph 1: Regression Analysis of the ratio between results obtained at time zero and at different time with 4 g / ml of the antibody – CD3 OKT3



Graph 2: Regression Analysis of the ratio between results obtained at time zero and at different time with 0,02 µg / ml of the antibody – CD3 OKT3



The stability programme has been continued after the first lots evaluated and we confirmed the results that the intermediate product Cryopreserved mDC is stable until 24 months of storage. Obtained data are summarized in the respective tables: Tab. 18- Stability programme: Vitality and purity, Tab.19 – Stability programme: Cell Phenotype, Graph 3.

Table 18: Stability programme: Vitality and purity

Batch: V-OP0147-DC034(A3)	VITALITY (%)	PURITY (%)	COMPLIANT
Fresh	94	63	yes
Thawed 28a week	90	67	yes
Thawed 61a week	84	61	yes
Thawed 86a week	82	62	yes
Thawed 117a week	88	63	yes

Batch: V-OP0270-DC047(A1)	VITALITY (%)	PURITY (%)	COMPLIANT
Fresh	96	60	yes
Thawed 29a week	92	64	yes
Thawed 59a week	88	67	yes
Thawed 78a week	92	77	yes
Thawed 107a week	89	68	yes

Batch: V-OP0233-DC062(A1)	VITALITY (%)	PURITY (%)	COMPLIANT
Fresh	94	64	yes
Thawed 32a week	94	62	yes
Thawed 58a week	95	62	yes
Thawed 84a week	94.8	63.3	yes
Thawed 111a week	95	62.5	yes

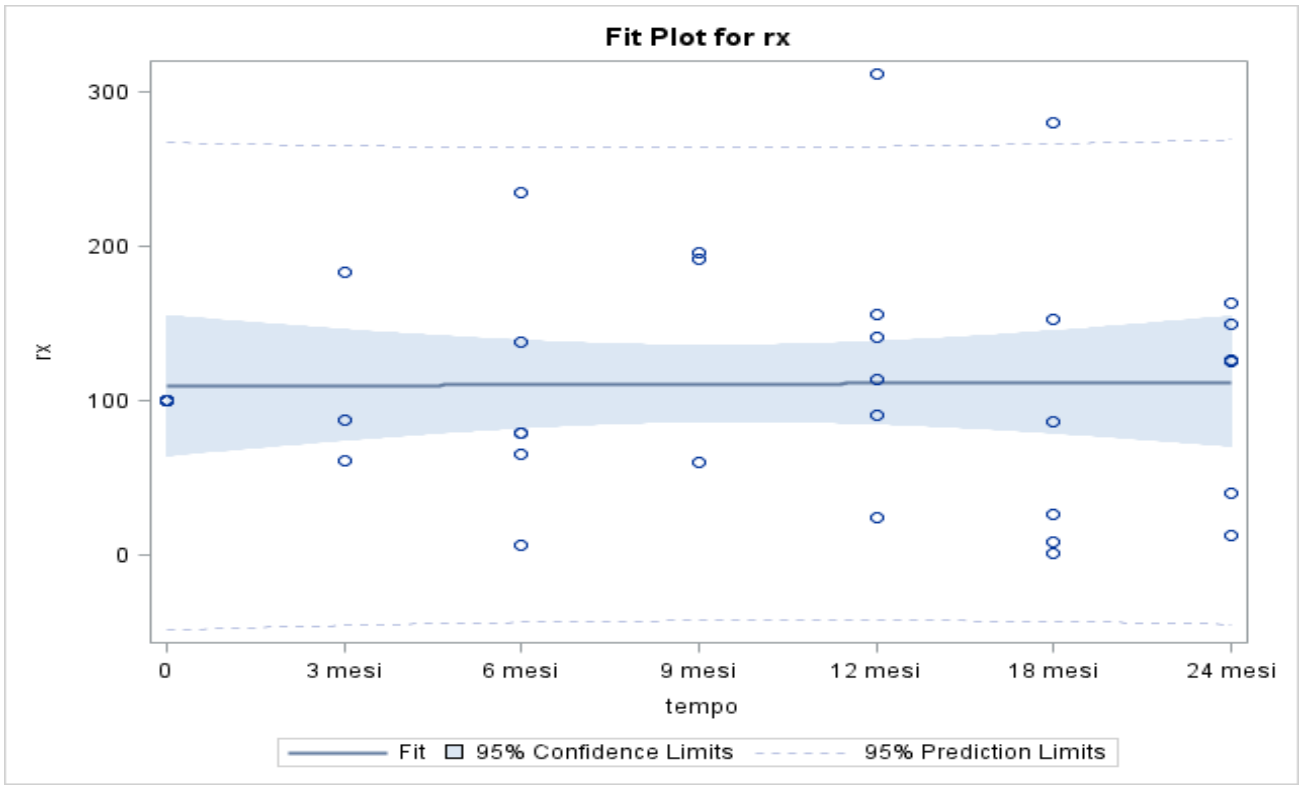
Table 19: Stability programme: Cell Phenotype

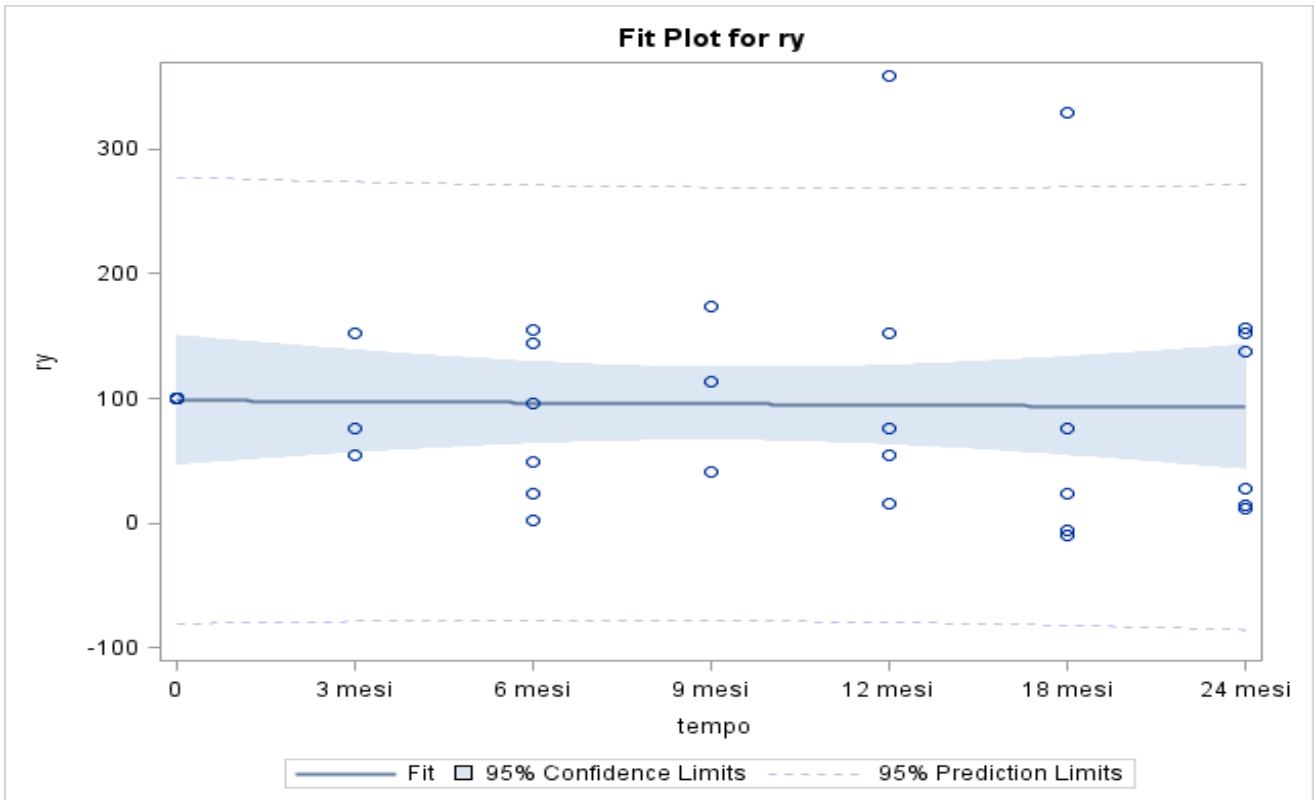
Batch V-OP0147-DC034(A3)	HLA-DR (%)	CD80 (%)	CD86 (%)	CD83 (%)	Compliant
Fresh	72	100	99	95	yes
Thawed 28a week	84	87	87	94	yes
Thawed 61a week	94	99	88	95	yes
Thawed 86a week	87	99	99	93	yes
Thawed 117a week	86	98	96	94	yes

Batch V-OP0270-DC047(A1)	HLA-DR (%)	CD80 (%)	CD86 (%)	CD83 (%)	Compliant
Fresh	68	86	95	70	yes
Thawed 29a week	85	92	91	60	yes
Thawed 59a week	85	97	94	58	yes
Thawed 78a week	79	99	94.5	60	yes
Thawed 107a week	86	98	96	94	yes

Batch V-OP0233-DC062(A1)	HLA-DR (%)	CD80 (%)	CD86 (%)	CD83 (%)	Compliant
Fresh	90	99	99	83	yes
Thawed 32a week	96	95	99	81	yes
Thawed 58a week	91	100	100	84	yes
Thawed 84a week	89.8	95.5	93.1	80.5	yes
Thawed 111a week	74.4	79.7	66.9	82.5	yes

Graph 3: Stability programme: Regression Analysis of the ratio between results obtained from 6 lots at time zero and at different time with 4 µg / ml and 0,02 µg / ml of the antibody –CD3 OKT3.





Costim Potency assay was upgraded with the check of the cell proliferation; this assay was qualified. CD3+ cells are labeled with PKH67 Green Fluorescent Cell Linker Midi Kit. In a U-bottom 96-well plate, $1x10^4$ live DCs and $1x10^5$ live T cells are co-cultured in triplicate (*background*). Moreover, the same amount of cells were seeded with 0.005 µg/ml OKT-3 (*COSTIM*). In every plate a positive control consisting of T cells and phytohemagglutinin-L and a negative control consisting of T cells and OKT-3 (0.005 µg/ml) was included. The co-culture was performed for 68 h at 37° C in a humidified atmosphere. At the end of incubation, cells were harvested and analysed by flow cytometry.

The stability of the formulated product in syringe, obtained from cryopreserved vaccine aliquots, has been performed as well. The results confirm the maintainance of cell vitality in syringe for at least 2 hours.
Drug product obtained from intermediate product Cryopreserved mDC or directly from apheresis product are comparable in terms of vitality, purity, phenotype characteristic (HLA-DR, CD80, CD86, CD83), sterility and function.

Extension of the shelf life of the Drug Product

Since the Drug Product can be administered in clinical centers located far from the manufacturing site, additional stability studies have been performed in order to evaluate the shelf life at 2-8°C for:

- Apheresis (starting material, WBC)
- Drug Product (finished product)

The study aim to extend the shelf life previously defined for the starting material and for the finished product, in order to allow the performance of multicentric clinical studies (see table 20).

Product	Previous shelf life	New shelf life (Ref. RC22)
Apheresis (starting material, WBC)	Not defined, freshly used	24 hours from the collection
Drug Product (finished product)	2 hours at RT	2 hours at RT 8 hours at 2-8°C

Table 20: Drug product and starting materials shelf-life extension

Results are summarized in the tables 21 and 22.

Table 21: Vitality – Purity – Phenotype of dendritic cells obtained from buffy coats treated 24 hours after donation

Acceptability limits: vitality > 70%, purity > 60% Cell Phenotype: HLA-DR > 60%, CD80 > 50%, CD86 > 60%, CD83> 40%							
Buffy coat n°	Vitalità %	Purezza %	HLA-DR %	CD80%	CD86%	CD83%	**Compliant**
I071517051516	97	68	93	92	98	90	yes
I071517051517	97	63	95	97	99	96	yes
I071517051700	95	64	91	98	83	45	yes

Table 22: Check on finished product thawed after 8 hours of storage:
Sterility – Endotoxin – Mycoplasma – phenotype – potency

Acceptability limits: vitality > 70% Cell Phenotype: HLA-DR > 60%, CD80 > 50%, CD86 > 60%, CD83> 40%						
Batch n°	Vitalità %	HLA-DR %	CD80%	CD86%	CD83%	Compliant
V-OP0174-DC039(A1)S2	91	84	98	98	84	yes
V-OP0176DC040(A1)S16	91	99	99	99	72	yes
V-OP0102-DC046(A1)S7	76	97	98	98	75	yes

Acceptability limits: Sterility: Sterile Endotoxin: ≤ 0.5EU/ml Mycoplasma: Absent Batches in which the ratios between X / X0 and Y / Y0 do not fall below 70% are considered stable. *Note:* *X, Y = stimulatory capacity of each sample after 8 hours of storage on healthy donor lymphocytes, in the presence of two different concentrations of OKT3 (4µg / ml and 0.02µg / ml)* *X0, Y0 = stimulatory capacity of each sample before storage on healthy donor lymphocytes, in the presence of two different concentrations of OKT3 (4µg / ml and 0.02µg / ml)*					
Batch n°	Sterility	Endotoxin	Mycoplasma	% X/X0	% Y/Y0
V-OP0174-DC039(A1)S2	Sterile	<0,1 EU/ml	absent	210	144
V-OP0176DC040(A1)S16	Sterile	<0,1 EU/ml	absent	208	198
V-OP0102-DC046(A1)S7	Sterile	<0,1 EU/ml	absent	197	146

2.1.A APPENDICES

2.1.A.1FACILITIES AND EQUIPMENTS

The "IGTF, IRCCS IRST" (Cell Factory) is a dedicated facility for the preparation of cellular therapy products for patients' treatment under clinical protocols. The Cell Factory has been authorized for the production of cellular therapy products (Autorizzazione AIFA n° aM-55/2012, 27/04/2012) and visited for GMP general revision. It is node of the Emilia Romagna Cell factories network (Deliberation of Giunta della Regione Emilia Romagna n°311, 23/03/2009).

The Cell Factory has a surface of 81 m^2, and is divided into two class B production areas (rooms 39-40) and one classe C area (room 37), which is accessed through a class D area (see enclosed layout). Different class areas are connected by filter zones. Classe A hoods are located within class B rooms, both equipped with continuous particle counters. All material enter and leave out the Cell Factory through S.A.S. provided with separate in and out boxes.

Quality Control Laboratory and Ambiental Parameter Monitoring Laboratory are adjacent to the Cell Factory. Stock room for acceptance and storage of reagents and disposables (it also includes +4°C, - 20°C and -80°C refrigerators) is located at room 59 (basement floor). The stock room is directly accessed by an elevator in the TCS Lab.

2.1.A.2ADVENTITIOUS AGENTS SAFETY EVALUATION

The process validation protocol has been extended to comprise the analysis of adventitious viruses on three consecutive lots of drug product prepared directly from apheresis product. Just before the final formulation of the product, surnatant from cultures of mature dendritic cells has been collected, analyzed for the presence of CMV, EBV, HSV1, HSV2 and Adenovirus. Results obtained showed that the process method systematically allows to obtain formulated product free of adventitious virus contamination (Table 23).

	BATCHES		
Viruses	**V-L012-DC03(A1)1**	**V-OP021-DC04(A1)2**	**V-OP026-DC05 (A1)1**
CMV	Negative	Negative	Negative
EBV *	Negative	Negative	Negative
HSV1	Negative	Negative	Negative
HSV2	Negative	Negative	Negative
Adenovirus	Negative	Negative	Negative

Table 23: Analysis of adventitious viruses on three consecutive lots of drug product.

* EBV was evaluated after sample's treatment with DNAse to eliminate the possible detection of virus related, to dead cell with a latent infection.

The results were confirmed in 2016 analysing five different lots related to four different patients, to monitor the adventitious virus contamination.

Updated data are shown in Table 24.

		Viruses				
Production Year	Batches	CMV	EBV*	HSV1	HSV2	Adenovirus
2013	V-OP0129-DC026(A1)	Neg.	Neg.	Neg.	Neg.	Neg.
2014	V-OP0129BIS-DC026(A2)	Neg.	Neg.	Neg.	Neg.	Neg.
2015	V-OP0147-DC034(A1)	Neg.	Neg.	Neg.	Neg.	Neg.
2015	V-OP0157-DC035(A1)	Neg.	Neg.	Neg.	Neg.	Neg.
2016	V-OP0156-DC036(A1)	Neg.	Neg.	Neg.	Neg.	Neg.
2017	Vf-OP0188-DC045(A1)1	Neg.	Neg.	Neg.	Neg.	Neg.
2018	Vf-OP0233-DC062(A1)1 Vf-OP0242-DC065(A1)1	Neg.	Neg.	Neg.	Neg.	Neg.
2019	Vf-OP0259-DC069(A1)1 Vf-OP0260-DC068(A1)1 Vf-OP0257-DC067(A1)1 Vf-OP0256-DC066(A1)1	Neg.	Neg.	Neg.	Neg.	Neg.
2020	Vf-OP0284-DC080(A1) Vf-OP0286-DC081(A1) Vf-OP0274-DC076(A1) Vf-OP0272a-DC074(A1)	Neg.	Neg.	Neg.	Neg.	Neg.

Table 24: Analysis of adventitious viruses on five lots produced in the period 2013 – 2020.

* EBV was evaluated after sample's treatment with DNAse to eliminate the possible detection of virus related, to dead cell with a latent infection.

2.1.A.3NOVEL EXCIPIENTS

Not applicable.

2.1.A.4SOLVENTS FOR RECONSTITUTION AND DILUENTS

Not applicable.

2. NON-CLINICAL PHARMACOLOGY, PHARMACOKINETICS AND TOXICOLOGY

For the summaries of non-clinical studies on the product "Autologous dendritic cell vaccine loaded with autologous tumor homogenate or lysate", reference is made to the Investigators Brochure which is an integral part of the clinical protocols.

2.3 CLINICAL DATA

For the summaries of all clinical data on the product "Autologous dendritic cell vaccine loaded with autologous tumor homogenate or lysate", reference is made to the Investigators Brochure, which is an integral part of the clinical protocols.

2.4 OVERALL RISK AND BENEFIT ASSESSMENT

Relevant data on clinical efficacy and safety of the product "Autologous dendritic cell vaccine loaded with autologous tumor homogenate or lysate" are provided in the Investigators Brochure.

In summary, treatment with this product, alone or in combination with radiotherapy or preleukapheresis interferon-alfa, resulted in a significant clinical benefit for patients showing positive immunization against tumor antigens (as displayed by delayed type hypersensitivity testing and ELISPOT data), without relevant toxicity, excepting local reaction in the vaccine administration sites and AEs related to IL-2 administration (given as immunological adjuvant after vaccine administration). Therefore, these findings strongly suggest an acceptable benefit to risk ratio.

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