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A clear view in closed isolators for autologous cell therapy

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Introduction

Autologous Cell Therapy is the paragon of what anyone could expect in case of cell disorder: individual blood collection, cells separation and cells modification before to inject to the patient who is the donor the repaired cells. The details of the working process to succeed is detailed here and have been described by ISPE.[1] (Figure 1)

T cells are collected from a patient: T cells are collected via apheresis, a procedure during which blood is withdrawn from the body and one or more blood components (such as plasma, platelets or white blood cells) are removed. The remaining blood is then returned to the body.

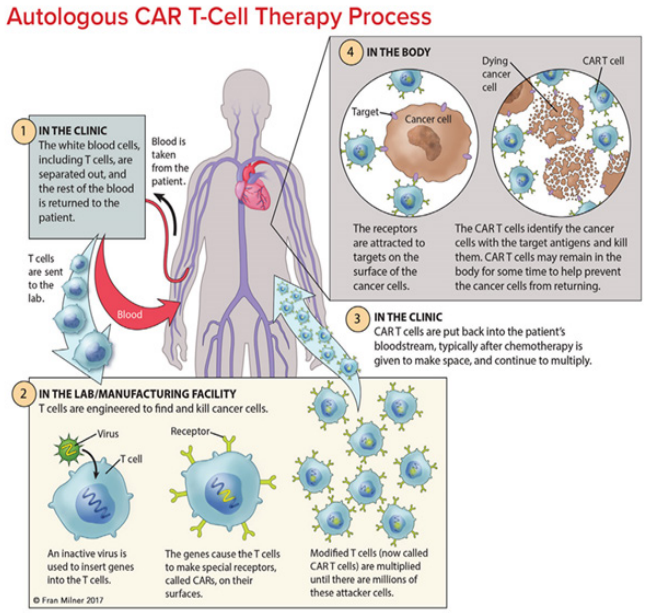

Figure 1 Autologous CAR T- Cell Therapy.

T cells are reengineered in a laboratory: The T cells are sent to a laboratory or a drug manufacturing facility where they are genetically engineered, by introducing DNA into them, to produce chimeric antigen receptors (CARs) on the surface of the cells.

After this reengineering, the T cells are known as "chimeric antigen receptor (CAR) T cells." CARs are proteins that allow the T cells to recognize an antigen on targeted tumor cells.

The reengineered CAR T cells are then multiplied: The number of the patient's genetically modified T cells is "expanded" by growing cells in the laboratory. This takes about 3 to 4 weeks. When there are enough of them, these CAR T cells are frozen and sent to the hospital or center where the patient is being treated.

At the hospital or treatment center, the CAR T cells are thawed and then infused into the patient: Many patients are given a brief course of one or more chemotherapy drugs to reduce the number of normal t cells in the body before they receive the infusion of CAR T cells. This is called "lymphodepletion," and it makes space for the new CAR T cells. The new CAR T cells are infused into the patient's bloodstream by IV or through an existing central line. This process takes less than 30 minutes. The CAR T cells that have been returned to the patient's bloodstream multiply in number. These are the "attacker" cells that will recognize attack and kill cells that have the target antigen on their surface.

The environment around a strict sterile practice before the final infusion in which these handlings must be done must follow the current Good Manufacturing Practices (cGMP) meaning that beyond the research and development there must be logic of producer to implement an installation fitting with regulations from both sides of the Atlantic Ocean.[2–3]

There are 2 choices of environment to isolate an aseptic bio-pharmaceutical process from its immediate surroundings either A in B or isolator as in the EMA revised Annex 1 in C or D. These A, B, C and D classes mean not only a defined ventilation/filtration, a speed of air and a filtered air renewal but a specific gowning for the personnel with times for gowning. For the comfort of the operator and the efficacy of the segregation personnel/process the choice of isolator with a class C or D surrounding according to local regulation eases the process and brings a full segregation operators/environment. It seems that there is a preference of working in a cleanroom Class C with a Class D surrounding.[4]

Integrated isolator for Cell Therapy with lock chamber for transfer of components, incubator and centrifuge has been presented to the market in 2016.[5] This type of equipment fits perfectly for the research and development of sequences to be then apply afterwards in daily routine.

In case of multiple Cell Therapy (CT) process each individual protocol must be separated and autonomous.

A solution is the use of individual flexible mini isolator (one per patient), which is a variation of what has been done at INRA (French National Institute for Agronomical Research) in the study of the microbiome of monoxenic and germ free mice. (Figure 2)

Figure 2

To keep individual separated process, we propose flexible sterile closed isolators, single and ready to use (SUT), which are Gamma sterilized. Closed isolators have been described and used from the late 40s in sterile surgery[6] and in breeding of lab germ-free rodents.[7]

All items and materials to be introduced in the Class C or D are bar coded or equivalent, sterile and double wrapped before to go through a H_2O_2 decontamination lock chamber after removing the first wrapping.

The cells coming from the outside of the Class C could be frozen at minus 70° C. Their entrance in the Class C clean room is done through an Alpha/Beta RTP transfer system (Figure 3). Which fits with the entrance door of the individual enclosure as workstation for the cells as a Cell Individual Process Closed Isolator -CIPCI®

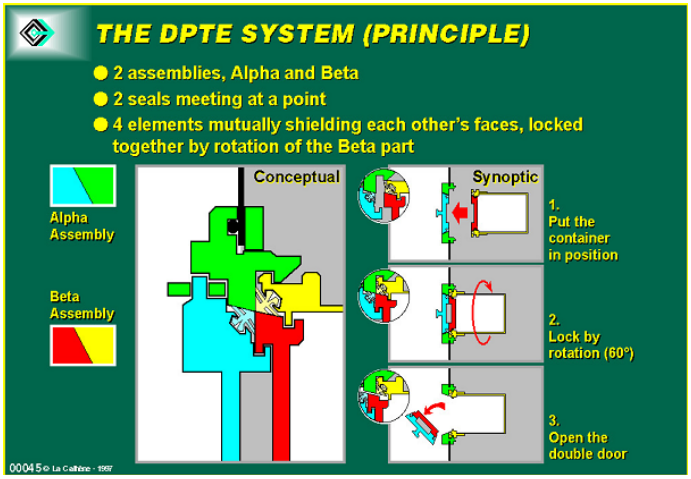

Figure 3 The DPTE system.

The CIPCI® is a transparent flexible positive pressure disposable sterile ready to use mini- isolator of approximate150 to 250 liters volume with forearms handling and a 190 MW RTP Beta door to be connected to the entrance Alpha door for the cells transfer to the storage isolator and to the service isolator for the storage and loading of the required items to allow the operator to proceed. The ventilation/filtration system of each of these isolators is composed of an inlet 0,5-micron hydrophobic membrane filter and an outlet HEPA filter. The gas used to inflate the isolator can be either regular compressed air or any combination of compressed gas to increase the development of the cells (added CO_2 for instance). The positive pressure is in the range of 30 to 50 Pa.

Key point in this configuration is the use of Alpha/Beta RTP systems for various loading and unloading without any risk of cross contamination from viable and non-viable particulates. This type of connection with rotation is QC/QA by their suppliers for the quality of the gaskets and the safety of the multiple connections.[6–7]

Each of this CIPCI® includes genderless CPC transfer connectors for the sterile transfer of liquids from the leukapheresis to the final patient's infusion. The top of each of these mini isolators offers a glass transparency for the microscope or camera which stays outside.

In case of the use of a camera AI could be used for the sorting of the cells. Main point about this very installation is the only use of "closed isolator systems" from the leukapheresis to the final infusion to the patient including the microscope/camera phase as described in (4a): "Excluded external contamination from the isolator's interior by transfer material via aseptic connections to auxiliary equipment rather than using openings to the surrounding environment. Closed systems remain sealed throughout operations" which is a warranty against any cross contamination with the surroundings.

Other important points to consider being in accordance with the User Requirement Specifications (URS) of most of the cell therapy process are:

- Cells transfer
- Thawing if necessary
- Centrifugation
- Incubation.

The cells are transferred directly to the CIPCI® from outside of the cleanroom through the MW RTP system. In the case of frozen cells at minus 70°C the CIPCI® includes a sleeve to be dipped in an external WFI water bath for the necessary time of thawing.

Ready to Use sterile MW RTP containers are used as centrifuge rotors allowing using off the shelf refrigerated or not centrifuge without any integration and modification.[8]

Incubation according to the flow of work can be done in an incubation room at 35°C as a part of the cleanroom or in a MW RTP container which must be placed in an appropriate incubator.

The whole system is under a computerized Data Integrity (DI) umbrella including the storage and the process with defined protocols and component lists to control the circulation of the cells and components from a to z knowing exactly at what stage are each of the cell process.

Both the storage isolator and the service isolator have a Unidirectional Flow ventilation/HEPA filtration to sweep away the quickest possible any trace of H_2O_2 during the aeration post bio-decontamination phase. The residual concentration of H_2O_2 is measured with a H_2O_2 Picarro® analyzer (Picarro, Inc. 3105 Patrick Henry Dr. Santa Clara, CA 95054 USA). The value of the H_2O_2 left level is part of the validation of the process.[9,10]

The storage isolator keeps sterile the necessary components and the service isolator (s) allow (s) the transfer to the targeted CIPCI® through its vertical (centrifuge bowls) and horizontal (components) MW RTPs.[11,12]

The turnkey set-up named ELECT® (Extensive Linked Enclosures for Cell Therapy) is sketched in Figure 4.

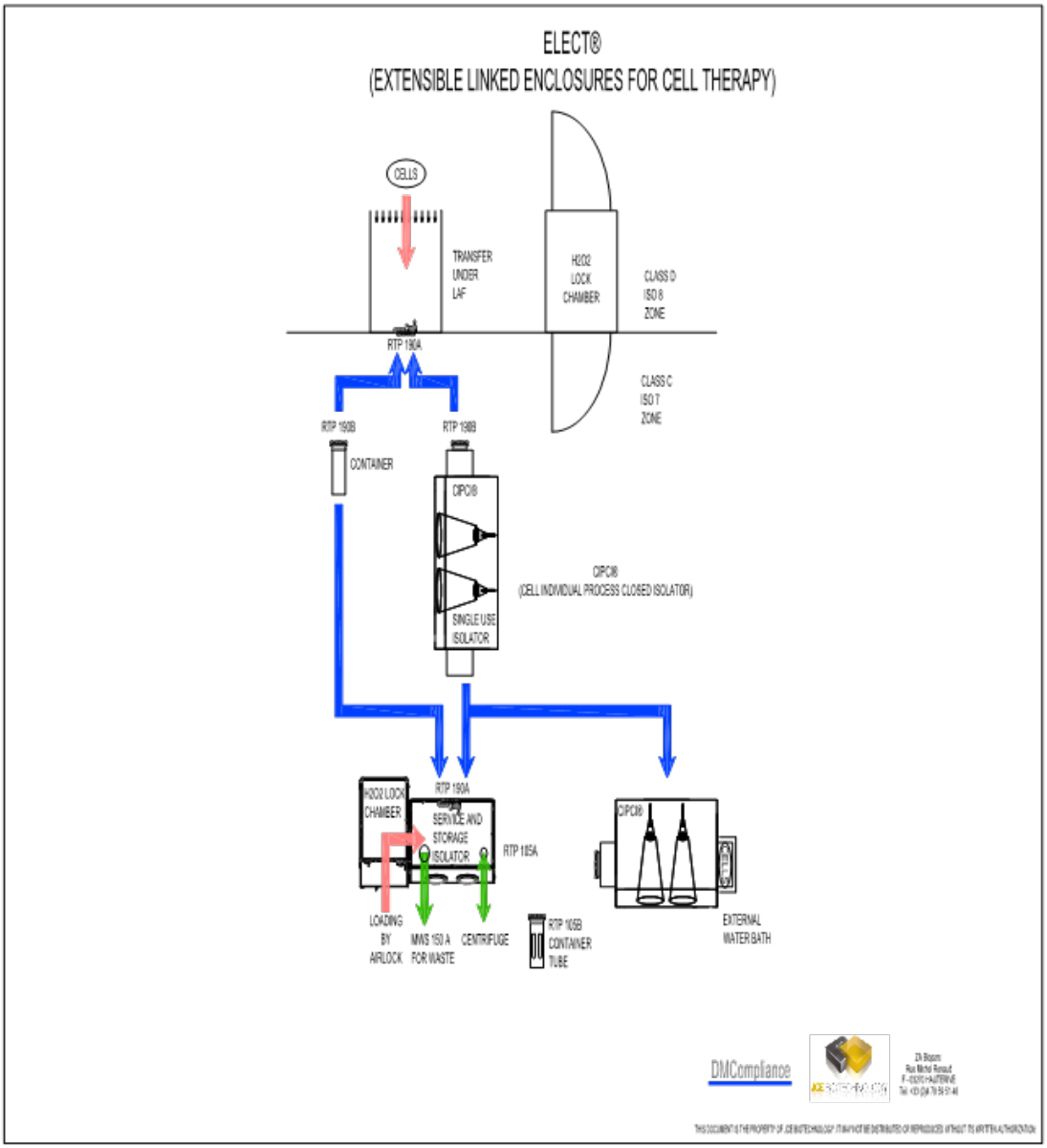

Figure 4 Extensive Linked Enclosures for Cell Therapy.

Within a ballroom architecture to get comfort for the personnel and a maximum patient safety at moderate Opex/Capex.

Conclusion

As a conclusion we face a gathering of technologies (isolator, microfiltration, HEPA filtration, VPHP, RTP, CPC connectors and SUT Gamma sterilization) which allows in accordance with the revised Annex 1 GMP compliance to proceed with strict individual protocols to the CART autologous therapies for solid tumors to cure patients in a pre-defined compliant timing and expense.

Acknowledgments

None.

Conflicts of interest

The author declares that they have no conflicts of interest.

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