

Summary

This dissertation deals with application of cryopreservation methods in the production and transport of advanced therapy medicinal products (ATMPs), including temporary storage in the Hospital cryostorage facility prior its clinical use. This work kept a strict adherence to European Union regulations and harmonized national legal norms.

The first section shows the method validation regarding the collection and cryopreservation of starting material - peripheral blood mononuclear cells for the production of registered ATMPs using chimeric antigen receptor of T-lymphocytes (CAR-T). As a practical output from this endeavor, we obtained the approval for the collection, processing, and distribution of mononuclear cells for ATMP manufacture, as well as an export license to foreign facilities where the final production of genetically modified ATMPs takes place.

The second part of this work presents the results of collection of starting material for ATMP production - bone marrow for manufacturing of human mesenchymal stromal cells (hMSCs)-based investigational ATMP and explains the development of the cryopreservation protocol for this investigational product under the conditions of good manufacturing practice (GMP) as an experimental part of the clinical trial EUDRA CT No. 2016-000926-21. The protocol was successfully used in all investigational products manufactured for the 6 patients included in the study, it could be used in the next clinical trials.

The third part deals with the toxicity of the cryoprotectant dimethyl sulfoxide (DMSO), which is currently used both in cryopreservation of starting materials for the ATMP manufacture and in cryopreservation of final products. In this section, I summarize the results of a retrospective study performed on a group of 13 patients in whom the hematologist indicated washing of DMSO from a thawed suspension of mobilized autologous cryopreserved peripheral blood progenitor cells immediately before their administration. This study shows that the removal of DMSO results in a significantly reduced cell viability, suggesting that this process should only be performed for high-risk patients.

In the last section of the dissertation I propose and verify the solution in practice for the storage of starting material, their transport to the manufacturer, temporary storage of ATMPs in the Hospital cryostorage facility, and transport of ATMPs to the Transplantation Unit in practice. The protocol hereby described met the pre-certification audit successfully, thus fulfilling the requirements for starting CAR-T therapy programme in University Hospital Hradec Králové.

Key words: Advanced therapy medicinal products

Cryopreservation

Cryoprotectans

Human mesenchymal stromal cells

Dimethyl sulfoxide

Good manufacturing practice