PROJECT

STEM CELL THERAPY FOLLOWING THANKSTEM SRL PROTOCOLS

THE HISTORY OF THANKSTEM SRL

Since 2006, Thankstem Srl has been dedicated to the development of autologous blood stem cell therapy .

Thanks to the in vitro qualification and quantification of cells at the University of Tor Vergata (Rome), Thankstem Srl is the sole owner of patents, approved in 34 countries, based on a safe and well-studied methodology that simplifies the acquisition of autologous stem cells at all stages of the process that uses:

• Autologous blood

• Whole blood

• Deprogramming substances in homeopathic quantity

• Time 72 hours

• Intravenous, intramuscular or subcutaneous local or general inoculation

• No logistics: just a kit consisting of 4-6 test tubes

Treatment Characteristics

Treatment consists in the administration in patients of non-induced autologous **pluripotent stem cells**, a cell type considered impossible to obtain in nature, using a simple blood sample taken from a peripheral blood vessel, just a few milliliters, which is then deprogrammed in special test tubes prepared in a "white chamber".

Our article "The plasticity of stem cells of blood origin: in vitro hepatic differentiation", published in The Journal of Cellular Physiolog , shows that the pluripotent stem cells which we obtain can

be reprogrammed into liver cells, which is impossible with mesenchymal or haematopoietic stem cells.

The stem cells we obtain, identified by Sorter and approved by some of the world's leading cellular clone experts, have only stem cell receptors that have been quantified and qualified, which allowed us to patent our system and make it suitable for a safe and legal human experimental trial.

Further advantages compared to other types of stem cells are the non-invasiveness of the blood sample (only a few milliliters of peripheral venous blood are sufficient) and the complete absence of side effects thanks to the correct application of our treatment protocol .

Autologous adult stem cells obtained from whole blood

The autologous plutripotenti adult stem cells were obtained from peripheral venous blood for de-programming by us.

The method has been patented in all its parts.

These cells have been identified through characterization effected by cytoflurometric analysis using Facsan Becton-Dickinson flow photometer and certified by cellular clone experts. Having only stem cell receptors they have been qualified and quantified, allowing us to patent our system.

We are dealing with **three types of adult stem cells**: **hematopoietic** stem cells , **mesenchymal** stem cells and **non-induced adult pluripotent** stem cells. The following work: " Blood-derived Stem Cells (BDSCs): in Vitro Hepatic Differentiation . "Journal of Cellular Physiology , 228: 1249-1254, Alaimo G, Cozzoli E, Marfe G, Esposito L, Ranalli M, Hmada D, Giordano A, Gambacurta A 2013 shows that some of the stem cells we obtain from the blood, the pluripotent stem cells, can be reprogrammed into hepatic cells, something that is impossible for mesenchymal or hematopoietic stem cells, but possible for stem cells with this characteristic.

The inoculation of stem cells, in addition to being autologous, is also homologous because the blood is present in every tissue. The patient’s autologous serum that is contained in the blood is used in the preparation to keep the cells alive thus avoiding the possibility of a Non-Self reaction.

The in vitro expansion method of peripheral venous blood stem cells enables non induced autologous pluripotent stem cells to be obtained, which once administered in adult mammals **do not give rise to side effects** such as reject or infection phenomena (they are not heterologous cells, are not kept in an animal culture medium) or develop teratomas (they are not retrovirus modified heterologous cells, Yamanaka technique) .

The method developed by Thankstem Srl allows autologous adult stem cells to be prepared in any medical structure avoiding any type of cellular manipulation made in the laboratory.

Indeed, there is no need to do treatments, such as the elimination of red blood cells or the purification of stem cells with respect to all other blood components or obtaining a greater quantity of pluripotent stem cells compared to the other two stem cell components, hematopoietic and mesenchymal stem cells, or culturing or differentiating into other cell types.

These additional treatments can stress the stem cells obtained, leaving them alive, but with less regenerative potential.

The preparation of stem cells according to our method avoids the complex laboratory preparation, allowing any hospital, clinic or doctor's surgery to prepare them by being in possession of a test tube kit enriched with deprogramming factors.

In other words, through a single test tube containing these factors, in which a sample of a few ml of peripheral venous blood with anticoagulant is put and left for 72 hours at a temperature of 4/8 degrees centigrade, it is possible to obtain not induced autologous pluripotent stem cells to improve orthopedic, cardiac, dermatological, ophthalmic, neurological, metabolic, etc., pathologies.

Further advantages compared to the other types of stem cells are the non-invasiveness of the sample extraction and the guarantee of a safe therapeutic protocol obtained from 50.000 inoculations over 10 years on animals close to humans in the zoological scale and on non provoked pathological pathogens. Furthermore, it was noted that the frequent application of this therapy for long periods (up to 10 years) in dozens of dogs and horses did not give rise to any side effects. .

SCIENTIFIC STEPS CARRIED OUT:

- 10 YEARS OF RESEARCH IN THE VETERINARY FIELD IN DOGS AND HORSES ON NON-PROVOKED PATHOLOGIES SIMILAR TO HUMAN. ONES(THE ONLY CASE OF A VIDEO PRESENT ON YOUTUBE AND FACEBOOK THAT SHOWS A BEFORE AND AFTER THERAPY , type: stem cells and polettini) ,
- 3 PATENTS DEPOSITED AND ACCEPTED IN 34 COUNTRIES AND A DEFINED THERAPEUTIC KNOWHOW ,
    - RM 2006A000498 “Expansion method for adult stem cells from blood, particulary peripheral blood, and relative application in medical field”;
    - UD 2008A000058 “Kit for collecting blood, preferably peripheral blood, for the production of stem cells”;
    - UD 2014A000075 “Method for expanding adult stem cells from whole blood”.
- SEVERAL STUDIES PUBLISHED IN MAJOR SCIENTIFIC MAGAZINES, PROVING THE THERAPY’S VALIDITY AND CONFIRMING THE PLURIPOTENT SPECIFICATIONS OF THE STEM CELLS OBTAINED. (page 5-6),
- THE SCIENTIFIC INSTITUTIONAL PROCEDURE IN ITALY FOR THE VALIDATION OF SUCH THERAPY IN DILATED CARDIOYMYOPATHY HAS BEEN STARTED THROUGH THE ADVISORY COMPANY AND CELL FACTORY (page 7-8-9-10).

THERAPY WILL BE CARRIED OUT ON:

- NEURODEGENERATIVE DISEASES (Alzheimer's, Parkinson's, Multiple Sclerosis ...)
- ORTHOPEDIC PATHOLOGIES (Tendinopathies Chondropathies, Arthritis, Traumas)
- ANTIAGING
- HEART DISEASE
- AUTOIMMUNITARY DISEASES
- LYME DISEASE
- OCULAR PATHOLOGIES.

Publications:

Polettini M, Zohar G, Gabbiani C, et al. “Application of stem cells obtained from peripheral blood in therapy of tendons, ligaments and skin’s injuries”, in Proceedings. 16th Annual Meeting of the Italian Association of Equine Practioners (SIVE) 2010;321-322

Polettini M, Cagni G, Gabbiani C, Zohar G and Gambacurta A. “Prospects of the use of stem cells obtained from peripheral blood in degenerative myocardial pathologies. Presented at : World conference on regenerative medicine. Leipzing Germany 2-4 november 2011

Spaas JH, Gambacurta A, Polettini M, et al. Purification and expansion of stem cells from 240 equine peripheral blood, with clinical applications. Vlaams Diergeneeskundig Tijdschrift. 241; 80:129-135 (2011)

Spaas J, Gambacurta A, Polettini M, Broeckx S, Van Hoeck F, De Schauwer C, Van de Walle G. Purification and expansion of stem cells from equine peripheral blood, with clinical applications. Vlaams Diergeneeskundig Tijdschrift, 2011, vol. 80; p. 129-135, ISSN: 0303-9021

Marfe G, Massaro-Giordano M, Ranalli M, Cozzoli E, Di Stefano C, Malafoglia V, Polettini M, Gambacurta A. “Blood Derived Stem Cells: An ameliorative therapy in ophthalmologic field. Journal of Cellular Physiology, March 2012, vol. 227, ISSN: 0021-9541, doi: 10.1002/jcp. 22953

Spaas, J., Broeckx, S., Van de Walle, G., & Polettini, M. (2012). The effects of equine peripheral blood stem cells (PBSC) on cutaneous wound healing : a clinical evaluation in four horses. CLINICAL AND EXPERIMENTAL DERMATOLOGY

Polettini M, Gambacurta A. Thankstem. Thank you stem cells! The peripheral blood stem cells cure, improve metabolism and quality of life. Therapeutic deduction by more than 1500 injections in horses and dogs. Edizioni Altea, Rome, Italy (2006)

Polettini M. Peripheral blood derived stem cells, treatment in touch with the future. Edizioni Altea, Rome, Italy (2011)

Polettini, “Blood Stem Cell Therapy for Eye Diseases in Horses” Edizioni Altea 2012

Poster presentation in NARMVA Congress, in Georgia, U.S.A, November 7-8-9, 2012

“How to use Stem Cells Obtained from Peripheral Blood in Dermatology and Ophthalmology” presented in Orlando at the Florida Association Equine Practitioners Congress in November 2010

“Prospects of the use of stem cells obtained from peripheral blood in degenerative myocardial pathologies” presented at the World conference on regenerative medicine, Leipzing Germany 2-4 november 2011

“The Role of the Horse Treating Vet” presented at Siena in “ Siena Cavalli Congress” in August 2012

Marfe G, Rotta G, De Martino L, Tafani M Fiorito F, Di Stefano C, Polettini M, Ranalli M Russo M.A., Gambacurta A. “A New Clinical Approach: Use of Blood Derived Stem Cells (BDSCs) for Superficial Flexor Tendon Injuries in Horses.” ELSEVIER Life Sciences 90 (2012) 825-830

Alaimo G, Cozzoli E, Marfe G, Esposito L, Ranalli M, Hmada D, Giordano A, Gambacurta A“ Blood-derived Stem Cells (BDSCs): in Vitro Hepatic Differentiation.” Journal of Cellular Physiology,228:1249-1254, 2013.

“Blood Derived Stem Cells: Why and How they work according to quantistic concepts in some horses and dogs’ pathologies irresponsive to treatments” presented at World Regenerative Medicine 2013 Congress in Leipzig.

“Regenerative Medicine: a New Theoretical and Clinical Approach” presented at Regenerative Medicine Veterinary Congress in Bonn February 2014

“The navicular syndrome: a new pathogenetic theory and therapy with blood stem cells” presented at Regenerative Medicine Veterinary Congress in Bonn February 2014

Polettini M.,Zohar G., gabbiani C.“The “non local” bio-physics effects in the Central Nervous System after treatment with stem cells obtained from de-programmed blood in veterinary clinical cases.” presented at the World conference on regenerative medicine, Leipzing Germany 21-23 October 2015.

Services of Accelera consisting in providing technical and regulatory consultancy for the preclinical development of a treatment based on autologous stem cells from peripheral blood, to be performed and guaranteed by Accelera preclinical senior leaders and specialists.

The requested services will include but without limitation:

- Available data review and gap analysis
- Support in defining a development strategy for autologous stem cells from peripheral blood.
- Technical supervision of preclinical efficacy studies.
- Design of preclinical safety studies to support Phase I clinical trials in humans.
- Supervision and contribution in documentation preparation
- Support in the preparation of Scientific Advice meeting(s) with Regulatory Authorities.
- Analysis of the residual concentration of factor still present in the blood sample at the end of the process will be measured by a commercial ELISA kit for human. If the evaluation on human BDSCs performed satisfactorily, confirmatory experiments will be conducted on dog peripheral blood

All the services are finalized at drafting and presenting the application to the italian health care institute (ISS).

Accelera shall provide Thankstem with a summary note of the tasks performed and the results thereof.

Human Blood Derived Stem Cells For he Autologous Treatment of Patients with Dilated Cardiomyopathy

Briefing Document for Pre-Submission ISS Consultation

PHASE I AND I/II CLINICAL SPERIMENTATION

1. ABBREVIATION LIST

BDSC	Blood Derived Stem Cell
DCM	Dilated cardiomyopathy
EGF	Epidermal Growth Factor
FGF-4	Fibroblast Growth Factor 4
GLP	Good Laboratory Practice
IGF-1	Insulin-like Growth Factor 1
IV	Intravenous

MSC	Mesenchimal Stem Cell
SC	Subcutaneous

2. RATIONALE

Dilated cardiomyopathy (DCM) is a progressive disease of heart muscle that is characterized by ventricular chamber enlargement and contractile dysfunction, sometimes associated with circulatory collapse, arrhythmias and thromboembolic events. The right ventricle may also be dilated and dysfunctional. Dilated cardiomyopathy is the third most common cause of heart failure and the most frequent reason for heart transplantation. The annual incidence of DCM is reported to be about 7 cases per 100,000 individuals.

In the past, the prognosis of DCM was considered extremely unfavorable with a mortality of 25-30% per year and a five-year survival rate of about 50%. In the last thirty years, the improvement of the knowledge on the chronic heart failure physiopathology, the early diagnosis, the use of validated pharmacological therapies (ACE inhibithors, beta blockers, diuretics) and of implantable cardioverter-defibrillators or artificial pacemakers, have drastically improved the long term outcome of DCM patients.

Currently, the annual incidence of major events (death or urgent heart transplantation) is <2% with an eight-year survival rate higher than 85%, while the annual incidence of sudden death is less than 0.5%.
Despite these encouraging data, a certain percentage of patients with a significant decline in functional parameters and ventricular remodeling was found in the long term, with significant relapses on the prognosis. For these reasons, the need for innovative therapies for DCM with greater long-term efficacy has been one of the areas in which scientific research has recently been focused.

One of the most recent experimental research concerns regenerative therapy with autologous mesenchymal stem cells (MSC) obtained from peripheral blood.
Mesenchymal stem cells were originally described by Fridenstein in the 1970s as a cell population with fibroblast like morphology, isolated from bone marrow, that were able to differentiate into osteoblasts, adipocytes and chondrocytes, and to support the growth of hematopoietic stem cells both *in vitro* and *in vivo*.
MSCs were identified in bone marrow, adipose tissue, placenta, amniotic fluid and umbilical cord blood. Usually present in limited numbers to be used for study or therapeutic purposes, the MSCs can be isolated and expanded in laboratory to reach a clinically relevant number.

The development of cell therapy and tissue engineering has made these cells of extreme interest with numerous possible applications and are being evaluated in numerous pre-clinical and clinical studies in various diseases, but especially in the regeneration of bone-cartilage, nervous and cardiac tissues.

In the veterinary field, the use of stem cells from adipose tissue or bone marrow has become in recent years a promising strategy for the treatment of diseases that do not respond to traditional therapies. Peripheral blood derived stem cells (BDSCs) are able to differentiate into a variety of cell types and may spontaneously repopulate different organs such as muscle, bone, liver or heart, and generate

differentiated cells as a response to acute or chronic damage. Because of these characteristics, the use of these stem cells in regenerative medicine appears to be very promising.
The efficacy of autologous treatment with BDSCs has been used in horses suffering from various pathologies (ophthalmic, tendon or cutaneous) with promising results. Local and /or intravenous administration of autologous BDSCs has been shown to significantly reduce inflammation in ophthalmic diseases or to repair tendon and skin lesions.

Experimental literature data show how pluripotent stem cells can be obtained from peripheral blood by a relatively simple method that uses factor to deprogram the blood cells. Stem cells of three different types can be obtained with this method: hematopoietic, mesenchymal and pluripotent. In a recent article published by Marfe et al., the phenotipic characterization of these cells using a FACSAria II cell sorter, has shown the expression of CD90, CD105 and CD117 receptors, normally expressed in hematopoietic and/or mesenchimal stem cells. In addition, a small fraction of cells also showed the presence of embryonic markers such as Sox2, Oct3/4 and Nanog.

Furthermore, it has been demonstrated that human BDSCs were able to differentiate into hepatic cells following incubation with different growth factors (EGF, IGF-1 and FGF-4). The cells obtained showed morphological and functional characteristic of hepatic cells and expressed functional markers such as α-fetoprotein.

THANKSTEM has patented a less complex method to obtain BDSCs that does not foresee the elimination of erythrocytes, the purification of stem cells and the incubation of cells for long periods. This method uses a kit of test tubes containing anticoagulant (Na citrate) or the deprogramming factor. At first, the peripheral blood is put into tubes containing Na citrate. The content of each tube is then transferred in another tube containing the deprogramming factor and maintained at 4-8°C for 72 hours before being re-inoculated.

This technique was used in the professional veterinary practice for the autologous treatment in total of 40 dogs, 18 females and 22 males of various breeds (mainly Doberman, Great dane and Newfoundland), suffering from dilated cardiomyopathy. The disease was divided by severity in 5 phases monitored through myocardial contractility at echocardiography. The de-programmed blood was inoculated after local asepsis subcutaneously in the cardiac area, in the perivertebral muscular area from the fifth to the tenth thoracic vertebrae and, in the most severe cases, also intravenously.

During the first 24 hours of the cell de-programming process, an oxygen ozone mixture can be added to eliminate possible endogenous infections of bacterial or viral origin. After treatment with autologous BDSCs a reduction of pathogenicity was obtained in 1-2 months in some of the treated dogs. In most cases there was an improvement and an increase in longevity compared to dogs with dilated cardiomyopathy reported in the literature. In younger animals, where the congenital component is greater, the result and longevity were lower than in adult animals. The treatment did not produce side effects and the dogs showed an increase of the survival period from twice to five times more than the average. The treatment was repeated approximately every 2/3 months for the first periods, then the injections were reduced to one every 6 months or one year. In some cases of Great Dane dogs for which the diagnosis was early, the treatment was performed annually for over six years. Drug therapy has never been interrupted in the first 2 years, but modified according to the needs. In some

cases after the first or after some inoculations, arrhythmias, such as atrial and ventricular fibrillations, have been no longer reported. The greater efficacy of stem cell therapy compared to the traditional one was shown in two Doberman puppies of the same litter that at one year showed symptoms of dilated cardiomyopathy and were treated: the first with the pharmacological therapy and the second with the pharmacological therapy in concomitance with BDSCs. Longevity was found to be one year longer in the second puppy.

Treatment with BDSCs also leads to a marked improvement in cardiac arrhythmias and in many cases even the complete disappearance. Another important result is the inhibition and reduction of side effects of the pharmacological therapy (diuretics, positive inotropes, ACE inhibitors and beta-adrenergic blockers) on the hepato-renal apparatus. In fact, the pharmacological therapy was continued in all cases treated but modified according to the evolution of the pathology. This allows a prolonged therapy and lengthens the estimated survival time.

Given the promising results of autologous therapy with BDSCs in dogs with dilated cardiomyopathy, a preclinical and clinical strategy has been developed to support the treatment of patients with dilated cardiomyopathy with autologous BDSCs obtained with the patented method from THANKSTEM.