

Abstract Presentation

Combined use of platelet gel and matrix modulating proteases (PROMOGRAN ®): a promising new synergy for the treatment of chronic skin wounds

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PURPOSE OF RESEARCH: The cronicity of a skin lesion is an important event as it greatly affects quality of life of patients and results in a significant burden of human and economic resources for medical treatment. The Regional Skin Bank and Center of Burns, in close synergy with Transfusion Medicine, has recently evaluated the clinical efficacy of autologous platelet gel and / or in combination with protease modulating matrix (PROMOGRAN) for the treatment of wounds. The aim of our study was to evaluate the clinical efficacy of treatment, in order to thoroughly investigate the clinical and biological features of this new combined treatment.

METHODS: We have been used for the preparation of platelet rich plasma (PRP) an autologous system of tubes patented certificate (My Cells ®) for obtaining PRP from a blood sample, then activated with thrombin and /or calcium gluconate. The platelet gel counterpart has been prepared in accordance with a system standard developed by the Transfusion Medicine. The matrix modulating protease (PROMOGRAN) is composed of 55% collagen and 45% of oxidized regenerated cellulose (Systagenix) and is a medication with advanced resorption after application it does not require removal. In this study it was used as a full support and / or fragmentation within the platelet gel according to the type of wound treated.

RESULTS AND CONCLUSIONS: All patients have responded positively and have never experienced side effects. We will present the most significant cases of patients showing the clinical results obtained in various steps of treatment until the recovers. Our initial experience suggests an actual clinical efficacy of autologous platelet gel and / or in combination with PROMOGRAN for the treatment of wounds. The addition of protease modulating matrix preparations, as well as to promote tissue regeneration, improves dramatically the quality of the final product, making it more compact and malleable for clinical application.

Vaccine gene therapy in metastatic renal cell cancer (MRCC)

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Aim and Introduction: current treatments in MRCC patients are unsatisfactory; in fact 5 years survival do not exceed 5%. Clinical response is around 7% in chemotherapy alone and 10-17% in combined treatment. Our aim was the evaluation of a treatment with immunotherapy and vaccine administration in MRCC patients using allogeneic tumour cell of renal origin transfected to produce interleukin-2 (IL-2) admixed to autologous tumour cells.

Materials and Methods: A human-IL2 expression vector, pcDNA-I Neo-IL2 (7683pb length), has been prepared by insertion of IL2-cDNA (683pb length) in HindIII/BamHI sites of the plasmid of the polylinker pcDNAINeo. The human IL2 cDNA (683 pb) was taken from pBC12 CMV/hIL2 plasmid and was obtained by double digestion with BamHI/HindIII. The fragment of 683 pb (cDNA IL2) purified on 1% agarose gel was linked to pcDNAINeo plasmid. The pcDNA/Neo also carries the neomycin gene under the control of the LTR of Rous Sarcoma Virus. ACHN tumour cell line has been transfected using CaPO₄ technique for obtaining an IL2 cells producing line. Cells were radiated to impede their replication. 3-10 millions of ACHN-transfected-radiated cells were periodically admixed to autologous tumour cells and injected subcutaneously.

Patients: 339 MRCC patients with multiple tumour sites were treated (grading G1-G4). 250-10.000 IL2 IU and 4-40 million of autologous LAK cells were monthly injected through the lymphatic vessel of the feet. IL2 was also administered by aerosol. Transfer Factor (3,5 IU, im, os) was monthly administered. 75 patients in progression disease were administered with vaccine after approval of our IRB and the central health authorities.

Results: *In vitro:* IL2 production of ACHN line was 278 pg/per million of cells/24h during 11-26 days after radiation (40GY). *In vivo:* scarce noticeable adverse side effects have been reported by IL2 and LAK cells

administration; they were completely absent due to the vaccine. The median overall survival in 339 was 66,6 months with significant difference in G2-G3 vs G4 patients. Median survival of the 75 pts vaccinated appeared significantly better (77,3 vs 61,9 $P < 0,01$) with respect to non vaccinated pts. Interesting difference, although not significant, has been also observed between patients treated with only allogeneic vaccine with respect pts administered autologous tumour cells. This observation, if confirmed, is particularly important being, the source of allogeneic vaccine, unlimited.

Bioengineering tissue in treatment of wounds: from basic research to clinical application

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PURPOSE OF RESEARCH: In recent years, thanks to the help of modern biotechnology, many natural and synthetic or biosynthetic skin substitutes, used increasingly for the treatment of wounds, have been marketed. However, there are substantial differences between scaffolds in terms of biocompatibility, structural and biological properties. The Burn Centre-Regional Skin Bank and Cell Factory, in collaboration with the Rizzoli Institute (Bologna, Italy), has developed a new patented (PCT/IB2008/002753) skin substitute with the main objective to use it as a cell scaffolds free to treat wounds involving bone-tendon structures.

METHODS: The scaffold is made from a minimum manipulation of dermis from multiorgan and/or multitissue donors, certificate suitable for transplantation according to current regulations. The skin tissue is subjected to a primary treatment to remove the cellular components present in the texture and responsible for the rejection of the recipient, then preserved by cryoserv to maintain the integrity of the architectural structure. The final processing of this tissue leads to obtain a new bioproducts which acts as a skin substitute.

RESULTS AND CONCLUSIONS: We will present the most significant cases of patients with difficult healing wounds admitted at our Burn Centre treated with the tissue cell free scaffold showing the clinical results obtained and those identified by the screening and histocitologyc analysis carried out on the fabric before graft and after transplantation at 7 and 30 days. The data presented show that the grafting / transplantation of scaffold cell free can take root on the receiver after only 7 days and be completely revascularized and repopulated by autologous cells of the patient. In all patients treated to date have never been observed adverse reaction, therefore, in our experience we can say that this is a replacement skin characterized by good biocompatibility, as well, as demonstrated by laboratory tests, to be essential for the biological properties clinical use, such as sterility, strength, malleability and preservation of vascular channels, collagen and elastic fibers.