An Optimized Method for Manufacturing a Clinical Scale Dendritic Cell-Based Vaccine for the Treatment of Glioblastoma

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Abstract

Immune-based treatments represent a promising new class of therapy designed to boost the immune system to specifically eradicate malignant cells. Immunotherapy may generate specific anti-tumor immune responses, and dendritic cells (DC), professional antigen-presenting cells, are widely used in experimental cancer immunotherapy. Several reports describe methods for the generation of mature, antigen-pulsed DC for clinical use. Improved quality and standardization are desirable to obtain GMP-compliant protocols. In this study we describe the generation of DC from 31 Glioblastoma (GB) patients starting from their monocytes isolated by immunomagnetic CD14 selection using the CliniMACS® device. Upon differentiation of CD14+ with IL-4 and GM-CSF, DC were induced to maturation with TNF-α, PGE2, IL-1β, and IL-6. Whole tumor lysate was obtained, for the first time, in a closed system using the semi-automated dissociator GentleMACS®. The yield of proteins improved by 130% compared to the manual dissociation method. Interestingly the Mean Fluorescence Intensity for CD83 increased significantly in DC pulsed with “new method” lysate compared to DC pulsed with “classical method” lysate. Our results indicate that immunomagnetic isolation of CD14+ monocytes using the CliniMACS® device and their pulsing with whole tumor lysate proteins is a suitable method for clinical-scale generation of high quality, functional DC under GMP-grade conditions.


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Introduction

Glioblastoma (GB) is one of the most aggressive forms of cancer and the most common primary malignancy in the central nervous system. Current treatment remains palliative [1,2], therefore novel therapies are greatly needed.

Numerous animal models indicate that Dendritic Cells (DC) are effective in the induction of therapeutic antitumor responses [3,4,5] and clinical trials indicate their efficacy in human pathologies [6,7] showing that effective immune responses within the CNS can be generated through the use of DC-based vaccines [8].

Immunotherapy with DC incubated with tumor lysate or peptides seems capable of generating a specific anti-tumor immune response [9,10], it is biologically safe without serious side effects noted in pre-clinical or clinical trials [11,12]. The development of methods to generate DC in accordance with good manufacturing practice (GMP) guidelines is mandatory [7]. Two phase-I clinical studies sponsored by the Istituto Neurologico Carlo Besta (DENDR1, EudraCT 2008-005035-15; DENDR2, EudraCT 2008-005038-62) for DC-based immunotherapy of GB have been approved by the Italian Ministry of Health.

Since circulating DC are few, representing only 0.1–1% of peripheral blood mononuclear cells (PBMC), large amounts of DC must be obtained in vitro from CD14+ monocytes purified from leukapheresis or buffycoat [13]. Differentiation of CD14+ into DC can be obtained in 7 days by utilizing GM-CSF and IL-4 [14]; DC can be induced to maturation in 24 h with a cocktail of pro-inflammatory cytokines [15].

Immune response specificity induced by DC is based on pulsing with tumor lysate that contains a multiple and unaltered spectrum of known and unknown tumor antigens which are patient-specific [16]. To ensure reproducible results in lysate preparation, we modified the tissue homogenization procedure for protein extraction using GentleMacS Dissociator, a closed system providing an increase in the yield of protein extraction.

The whole process is performed in the Clean-room facility of the “Cell Therapy Production Unit” in the Istituto Neurologico Carlo Besta.
Il Fondo di Gio

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