Reviewer's report

Title: A full scale comparative study of methods for generation of functional DCs for use as cancer vaccines

Version: 1 Date: 16 March 2007

Reviewer: Marc Dauer

Reviewer's report:

General

Based on a novel protocol for generation of monocyte-derived dendritic cells (DCs) in 48 hours established by Dauer et al. (FastDC; J Immunol. 2003 170(8):4069), the authors have performed a comparative analysis of the FastDC protocol with a standard 7-day protocol aimed at adaption for large-scale clinical use. They used elutriation to isolate monocytes from leukapheresis products and a closed culture system with sterile Teflon bags for DC generation. For antigen loading, the different DC preparations were transfected with whole tumor cell RNA by electroporation. Transfection efficacy, phenotype and T cell stimulatory capacity of FastDC and standard monocyte-derived DC were compared. The authors claim that the 48-hour FastDC protocol yields DC with equal quality and efficacy compared to a standard 7-day protocol and will be implemented in clinical trials. The experiments described are generally well designed and the results of considerable interest to the scientific community. The availability of a rapid and reliable protocol for large-scale generation of DCs may facilitate evaluation of DC-based tumor vaccination in clinical trials.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1) No statistical analysis has been performed for any of the experiments described. Although the value of statistical analysis for the kind of experiments described is debatable, the authors should at least provide information on how many independent experiments were performed for each of the results described (figures 2 and 3).

2) In the first paragraph of the results section the authors described phenotypic characteristics of FastDC that have already been reported by Dauer et al. (J Immunol. 2003 170(8):4069). This should be clearly stated and the respective citation referred to.

3) In the second paragraph of the results section, the authors state that FastDC retained a monocytic phenotype (expression of CD14, low expression of CD1a and CD209) even after 48 hours of culture with GM-CSF and IL-4. Dauer et al. have initially reported that monocytes develop an immature DC phenotype with complete downregulation of CD14 already after 24 hours of culture with GM-CSF and IL-4 (J Immunol. 2003 170(8):4069). This finding has been confirmed in subsequent studies by the same group (Dauer et al., J Immunol Methods 2005 302(1-2):145-55). These controversial findings should be discussed briefly. One possible explanation could be the comparably high concentration of GM-CSF used by the authors that may promote development of a monocytic/macrophage-like phenotype in their DC cultures. Alternatively, the different processing of monocytes due to the use of elutriation technique and Teflon culture bags may lead to enhanced early activation of the monocyte precursors thus preventing a more rapid DC differentiation.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) In the third paragraph of the results section, the authors present data from a so-called “wash-out” analysis, i.e. culture of DCs in the absence of cytokines or growth factors. They are able to show that FastDC retain their mature phenotype in the wash-out culture. This confirms previous findings by Dauer et al. described earlier (Dauer et al., J Leukoc Biol 2006 80(2):278-86). This should be briefly discussed and the respective citation included into the manuscript and referred to.

2) To avoid misunderstandings, the second paragraph of the results section should be entitled “Immunophenotype of FastDC and Standard DCs”.

3) The authors do not explain how the results on viability of DCs described in figure 2 were obtained. This should be clarified. Moreover, the graphic presentation of data in figure 2 should be improved. In the present form, data are not clearly visible and thus hard to interpret.

4) Throughout the manuscript, the different DC preparations (FastDC vs. standard monocyte-derived DCs) are termed differently, either Fast DC or Fast DCs as wells Standard DC or Standard DCs. Terminology should be standardized. Furthermore, the abbreviation “DCs” should be avoided in the title of the manuscript.

5) The manuscript deserves revision regarding correct use of the English language. There are several
grammatical and spelling errors throughout the manuscript.

Discretionary Revisions (which the author can choose to ignore)

1) The authors do not provide any data on the quality of the monocyte preparation that is obtained by the elutriation technique as compared to other techniques such as plastic adherence or MACS technology (e.g. purity, viability). The quality of monocyte isolation may influence the quality of the DC preparation obtained after culture with GM-CSF and IL-4.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.