New fully automated clinical-scale process for the enrichment of cross-presenting CD141 (BDCA-3)$^+$ dendritic cells

Jessica Heckötter, Tobias Ozimkowski, Mareke Brüning, Katja Petry, Emiliano Melandri, and Andreas Dzionek
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Introduction

CD8α$^+$ DCs in the mouse constitute a subpopulation with excellent cross-presentation capacity, which is essential for the initiation of effective CTL responses against tumors. An equivalent human subset of professional cross-priming DCs (XP-DCs) has recently been defined as lineage-negative, MHC class II^{high}, CD141$^+$, XCR-1$^+$, CLEC9A$^+$ DCs, also named DC2.[1–3] Their potential for cancer immunotherapy has not been explored so far due to their scarcity and the lack of appropriate cell isolation methods.
Here we describe a newly developed, fully automated process that enables enrichment of XP-DCs for clinical use. It relies on GMP reagents, dedicated single-use tubing sets, and software for the CliniMACS Prodigy® Instrument (fig. 1).
To optimize the cell isolation performance, monocytes, B cells, and T cells were depleted prior to the enrichment of XP-DCs. This two-step process resulted in a more than 1000-fold enrichment of target cells with high recovery and viability. Enriched cells were fully functional, acquired a mature phenotype, and produced large amounts of IFN-λ upon TLR 3 stimulation. For quality control and in-process control we developed a 7-color staining protocol that discriminates between the very rare XP-DCs and CD141^{low} monocytes, plasmacytoid DCs (pDCs), and CD1c (BDCA-1)$^+$ myeloid DCs (mDCs). Altogether, this new method enables for the first time the clinical-scale enrichment of XP-DCs, contributing to the development of next-generation DC vaccines.



Figure 1

Results

1 Workflow for the CD141 (BDCA-3) Enrichment System on the CliniMACS Prodigy® Instrument

The preparation of cells that are suitable for the development of XP-DC-based vaccines requires a two-step cell separation process. Using the tubing set CliniMACS Prodigy® TS 310 and the BDCA-3 Pre-depletion process, monocytes, B cells, and T cells are depleted efficiently in the first step. In the second step, utilizing the BDCA-3 Enrichment process and a second TS 310, XP-DCs are enriched by means of CliniMACS® CD141 (BDCA-3) GMP MicroBeads (fig. 2).

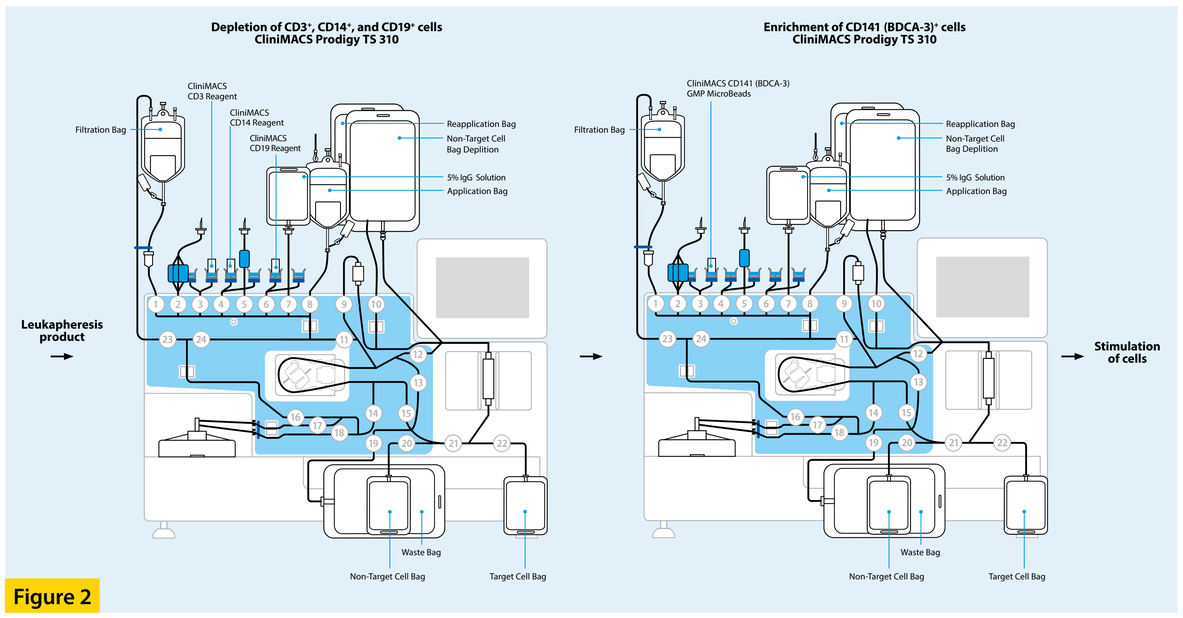


Figure 2

2 7-color staining protocol for in-process control of the clinical XP-DC separation

For the accurate analysis of the very rare CD141^{high} XP-DCs, a reliable staining protocol for identification of these cells and concurrent discrimination of CD141^{low} monocytes, pDCs, and mDCs is required.
After standard gating on viable CD45$^+$ leukocytes, the newly developed 7-color staining protocol allowed for discrimination between XP-DCs (CD141$^+$, CLEC9A$^+$) and monocytes (CD141^{low}, CD14$^+$), pDCs (CD141^{low}, BDCA-2$^+$, CD123$^+$), and mDCs (CD141^{low}, FCεRIα$^+$, CD123$^-$). The cell samples were stained with CD45-VioGreen™, CD14-PE-Vio® 770, CD370 (CLEC9A)-VioBright™ FITC, CD141 (BDCA-3)-APC, CD123-APC-Vio 770, CD303 (BDCA-2)-PE and Anti-FCεRIα-VioBlue® and analyzed using the MACSQuant® Analyzer 10 and MACSQuantify™ Software.

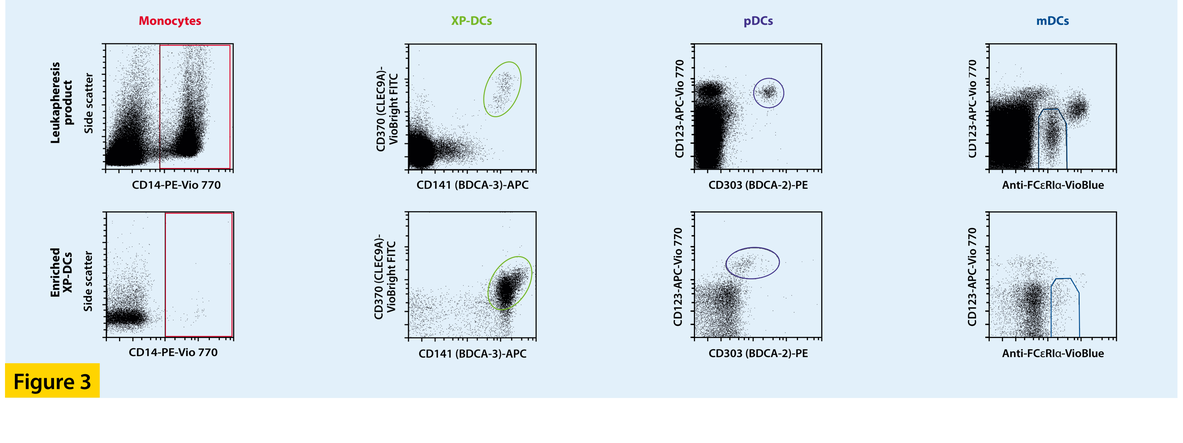


Figure 3

3 Performance of the CliniMACS Prodigy® CD141 (BDCA-3) Enrichment System

XP-DCs were enriched from leukapheresis samples by the above described two-step process (n = 18). As a result of the efficient pre-depletion process, monocytes, T cells, and B cells were depleted by more than 10,000-fold (fig. 4A).
XP-DCs were enriched to a mean purity of 60% starting from leukapheresis products with an average of 3.6×10^9 viable leukocytes and 0.07% XP-DCs (fig. 4B). XP-DCs were enriched with an average recovery of 51% (fig. 4C) which corresponded to an average XP-DC count of 3.9×10^5 per 1×10^9 total initial cells (fig. 4D). The target cell fraction contained a residual 9% CD141^{low} pDCs and 11% CD141^{low} mDCs on average (not shown), resulting in a CD141$^+$ cell purity of 80% on average (fig. 4b).

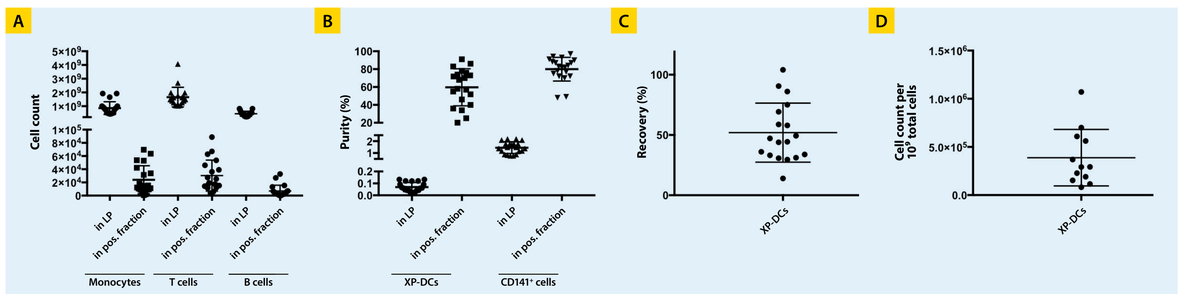


Figure 4

4 Enriched cells are fully functional, acquire a mature phenotype, and produce large amounts of IFN-λ

XP-DCs express high levels of TLR 3 and respond to poly-(I:C) stimulation by up-regulation of activation markers and production of high amounts of IFN-λ[4]. To test the activation status of the cells, XP-DC preparations were stimulated overnight at a cell density of 1×10^6 cells/mL in the presence of poly (I:C) (50 µg/mL; InvivoGen) and analyzed by flow cytometry (n = 4). Expression levels of activation markers CD80, CD83, and CD86 as well as MHC class I (HLA-ABC) and MHC class II (HLA-DR) molecules were significantly increased upon activation and showed a typical activation pattern (fig. 5A; black histogram: non-stimulated, red: stimulated; bar chart: n = 4). In addition, cell culture supernatants were analyzed for the secretion of IFN-λ by means of ELISA. Production of further cytokines was measured using the MACSPlex Cytokine 12 Kit, human. The stimulated cells secreted large amounts of IFN-λ, TNF-α and IL-6 upon TLR 3 triggering (fig. 5B). Substantial amounts of IFN-α could also be detected, presumably due to residual pDCs.

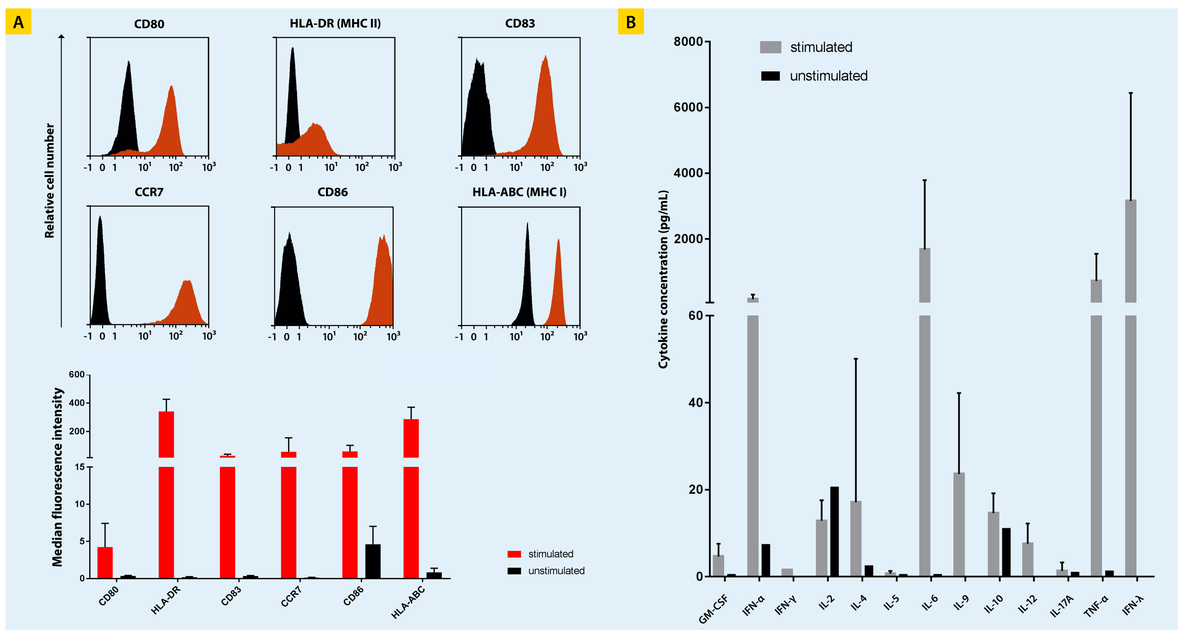


Figure 5

Conclusion and outlook

- The CliniMACS Prodigy CD141 (BDCA-3) Enrichment System allows for the isolation of CD141$^+$ DCs with high purities and recoveries.
- The newly developed 7-color staining protocol enables reliable quality control of the separation process.
- XP-DCs isolated using the CliniMACS Prodigy CD141 (BDCA-3) Enrichment System are fully functional, as they acquire a mature phenotype and produce large amounts of IFN-λ upon stimulation with TLR 3 agonists.
- The antigen uptake capacity of XP-DCs has been shown in preliminary experiments and is currently the subject of further investigations.

References
1. Robbins, S.H. *et al.* (2008) Genome Biol. 9: R17.
2. Poulin, L.F. *et al.* (2010) J. Exp. Med. 207: 1261–1271.
3. Bachem, A. *et al.* (2012) Front. Immunol. 3: 214.
4. Lauterbach, H. *et al.* (2010) J. Exp. Med. 207: 2703–2717.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635122.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. MACS® GMP Products are for research use and *ex vivo* cell culture processing only, and are not intended for human *in vivo* applications. For regulatory status in the USA, please contact your local representative. MACS GMP Products are manufactured and tested under a quality system certified to ISO 13485 and are in compliance with relevant GMP guidelines. They are designed following the recommendations of USP <1043> on ancillary materials. The CliniMACS® System components, including Reagents, Tubing Sets, Instruments, and PBS/EDTA Buffer, are designed, manufactured and tested under a quality system certified to ISO 13485.
In the EU, the CliniMACS System components are available as CE-marked medical devices for their respective intended use, unless otherwise stated. The CliniMACS Reagents and Biotin Conjugates are intended for *in vitro* use only and are not designated for therapeutic use or direct infusion into patients. The CliniMACS Reagents in combination with the CliniMACS System are intended to separate human cells. Miltenyi Biotec as the manufacturer of the CliniMACS System does not give any recommendations regarding the use of separated cells for therapeutic purposes and does not make any claims regarding a clinical benefit. For the manufacturing and use of target cells in humans the national legislation and regulations – e.g. for the EU the Directive 2004/23/EC ("human tissues and cells"), or the Directive 2002/98/EC ("human blood and blood components") – must be followed. Thus, any clinical application of the target cells is exclusively within the responsibility of the user of a CliniMACS System.
In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Sets TS and LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved; all other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE). CliniMACS MicroBeads are for research use only and not for human therapeutic or diagnostic use.
CliniMACS, CliniMACS Prodigy, MACS, the MACS logo, MACSQuant, MACSQuantify, Vio, VioBlue, VioBright, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec GmbH and/or its affiliates in various countries worldwide. Copyright © 2017 Miltenyi Biotec GmbH and/or its affiliates. All rights reserved.