StraightFrom® MicroBeads: Fast T cell isolation for CAR T cell manufacturing without density gradient centrifugation

Simon Lennartz, Dominik Lock, Carolin Kolbe, Gregor Winkels, Mario Assenmacher, Andrew Kaiser
Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany; *Miltenyi Biotec Inc., San Diego, USA

Scan the QR code to download the poster (PDF):

Introduction

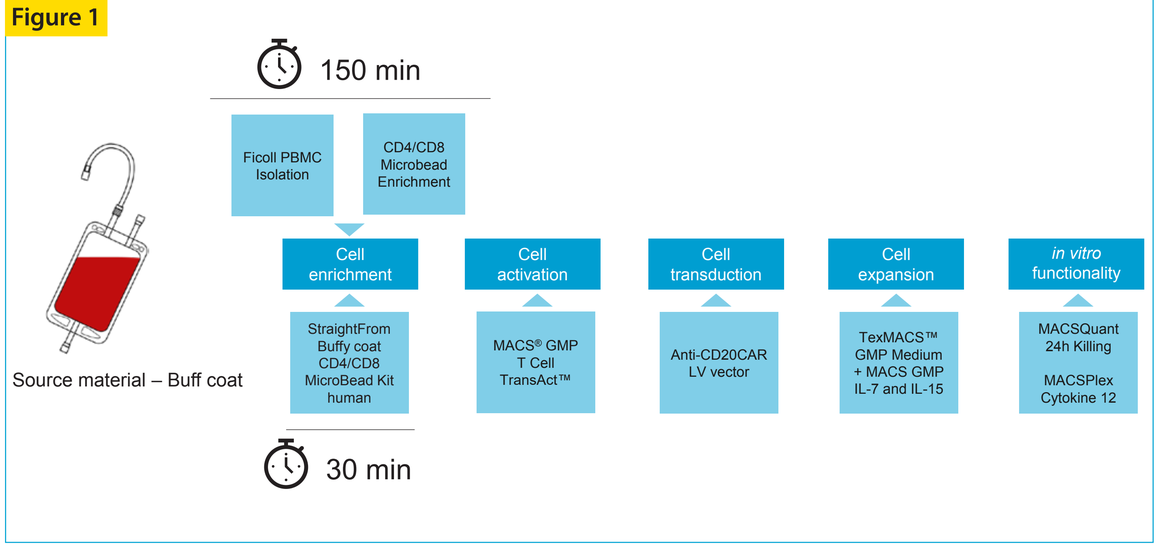
Adoptive immunotherapy using chimeric antigen receptor (CAR)-engineered T cells redirected to specifically eliminate cancer cells represents a powerful therapeutic tool for the treatment of hematological malignancies. However, clinical concepts based on such gene-modified T cells, although efficacious, have a high potential for improvements regarding efficacy as well as safety. Therefore, fast, efficient and flexible workflows are of utmost importance to develop novel CAR technologies. In this context, we established an optimized CAR T cell research workflow, combining cell isolation, culture and analysis tailored for CAR T cell generation.

In contrast to conventional protocols aiming to manufacture CAR T cells that require a time-consuming density gradient centrifugation to prepare PBMC, this step is dispensable when using StraightFrom® MicroBeads. It is thus possible to accelerate the experimental process by starting directly from whole blood, buffy coat and blood products.
Here, we compared the StraightFrom® MicroBeads approach with a conventional Ficoll protocol to manufacture CD20 CAR-expressing T cells and confirmed a comparable quality of both cellular products as reflected in similar CAR expression patterns as well as cytolytic potential *in vitro*.

Methods

1 Workflow overview

Figure 1

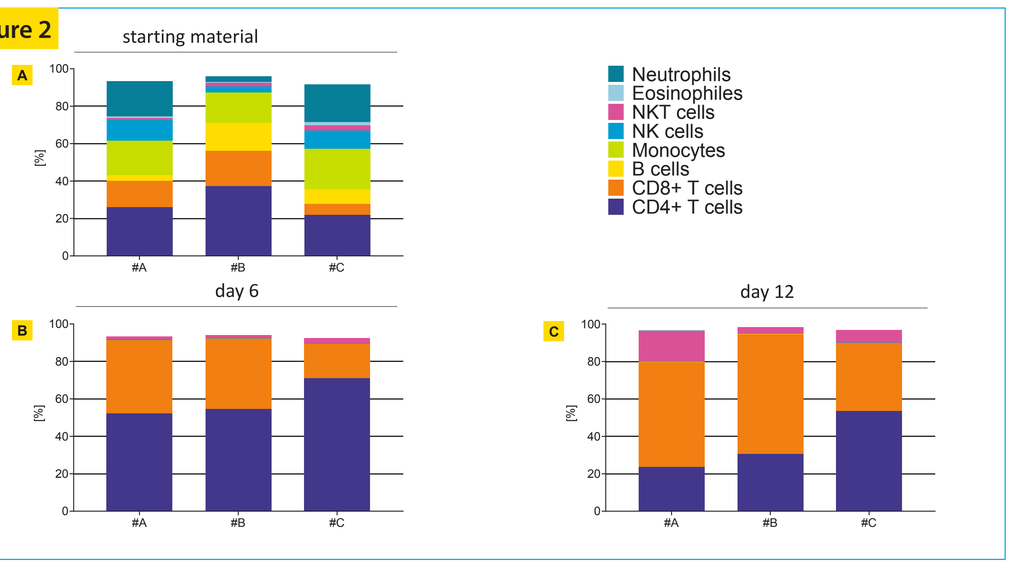


For the comparison of both protocols, buffy coat obtained from healthy donors were divided into two equal parts. When using StraightFrom®, one part of the buffy coat was stained with CD4/CD8 MicroBeads for 15 minutes at 2–8 °C. Subsequently, labeled CD4- and CD8-positive cells were separated directly using six Whole Blood columns placed in the magnetic field of a QuadroMACS™ Separator.
The other part of the buffy coat was used for a density gradient centrifugation using Ficoll Paque® PLUS density gradient media (GE Health-care) according to manufacturer's instructions to initially prepare PBMC. Thereafter, PBMC was stained with CliniMACS®CD4 and CD8 GMP MicroBeads for 15 minutes at 2–8 °C. Finally, labeled CD4- as well as CD8-positive T cells were isolated using two LS columns and a QuadroMACS™ Separator.
After enrichment, both protocols used identical conditions to further activate, transduce and expand the cellular products.

Results

1 Cell enrichment

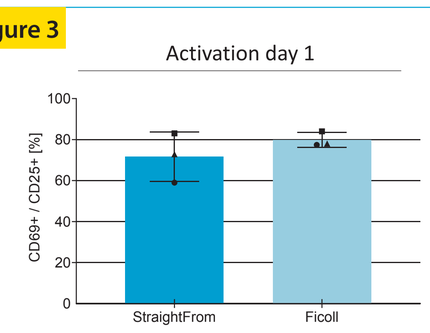
Figure 2



Cellular composition was analyzed by flow cytometry on the MACSQuant® Analyzer 10 via MACSExpress mode® at different time points.
Starting material of all three donors showed a comparable cellular composition including T cells, B cells, monocytes, NK cells, NKT cells as well as granulocytes (A). The enriched fractions were mainly composed of CD4 and CD8 T cells on day 6 (B) as well as on day 12 (C).

2 Activation status

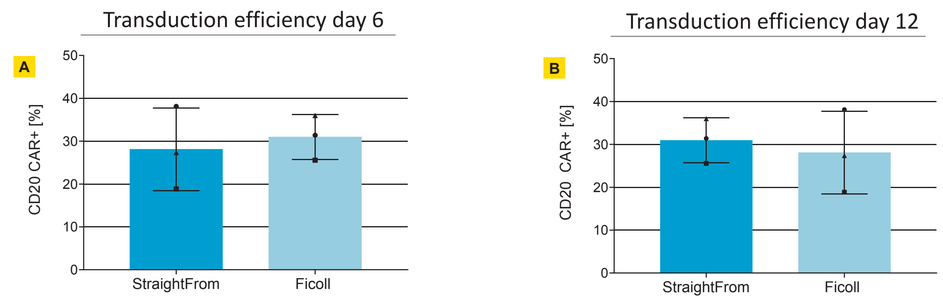
Figure 3



Enriched CD4/CD8 T cells of three donors were activated using MACS® GMP T Cell TransAct™ and cultured in TexMACS™ GMP Medium supplemented with MACS GMP Recombinant Human IL-7 and MACS GMP Recombinant Human IL-15. 24h after activation, both Ficoll- as well as Straight-From®-derived T cells showed a similar activation status as assessed by measuring the co-expression of CD25 and CD69.

3 Transduction efficiency

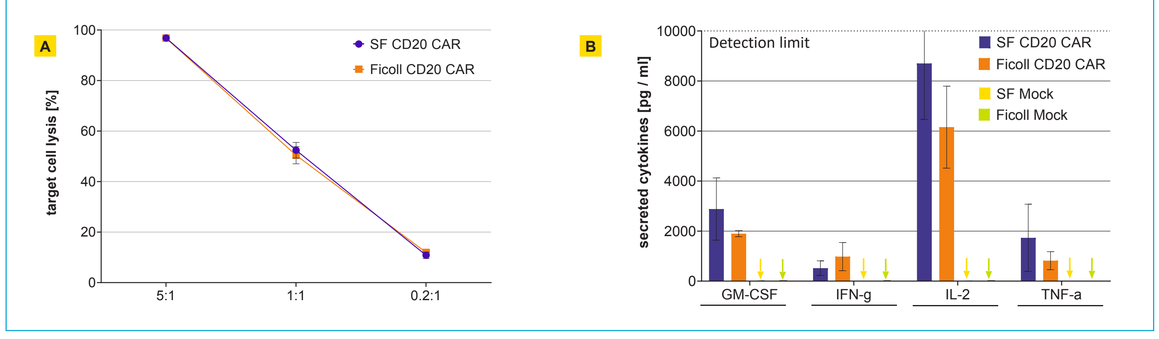
Figure 4



On day 1, T cells of each donor were transduced with equal amount (MOI=8) of lentiviral particles encoding a CD20-directed CAR.
Transduction efficiency was analyzed by flow cytometry using a CD20CAR detection reagent, anti-human (Miltenyi Biotec). Independently of the manufacturing protocol and donor, comparable transduction efficiencies were observed on day 6 (A) and on day 12 (B).

4 *In vitro* functionality

Figure 5



To assess and compare the cytolytic potential of CD20-directed CAR T cells, CD20-positive JeKo-1 cells were co-cultured either with T cells of three donors derived form StraightFrom®- or Ficoll-based protocols at different effector to target ratios for 20 hours.

Analyzing both target cells lysis (A) as well as release of proinflammatory cytokines of three donors (B) confirmed that no ascertainable differences exist with regard to the cytotoxic potential of both cellular products.

Conclusion

Here, two different manufacturing protocols for the generation of CAR T cells were compared.
This side by side comparison of the standard MACS® MicroBeads and the StraightFrom® MicroBead Kits revealed that regardless of the isolation approach, magnetic cell separation using MACS Technology ensures preservation of T cell functionality.
Using the innovative StraightFrom MicroBead solution to isolate CD4- and CD8-positive T cells directly from blood products allows the omission of density-gradient centrifugation for PBMC generation, thereby saving processing time of up to two hours and reducing user variability.
We observed comparable transduction efficiencies, activation status and cytolytic potential using the StraightFrom® approach.
In combination with options for automation, StraightFrom Technology is an ideal tool for streamlined and standardized experimental procedures.

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 as a Humanitarian Use Device (HUD), authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this indication has not been demonstrated. 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 logo, MACS MACSQuant, MACSQuantify, MACSxpress, the Miltenyi Biotec logo, QuadroMACS, StraightFrom, TexMACS, TransAct, VioBlue and VioGreen are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. Ficoll, Ficoll-Paque, and Percoll are trademarks of GE Healthcare companies. Cy is a registered trademark of GE Healthcare UK Limited. Leukopak is a registered trademark of StemExpress LLC.
All other trademarks mentioned in this document are the property of their respective owners and are used for identification purposes only. Copyright © 2021 Miltenyi Biotec and/or its affiliates. All rights reserved.