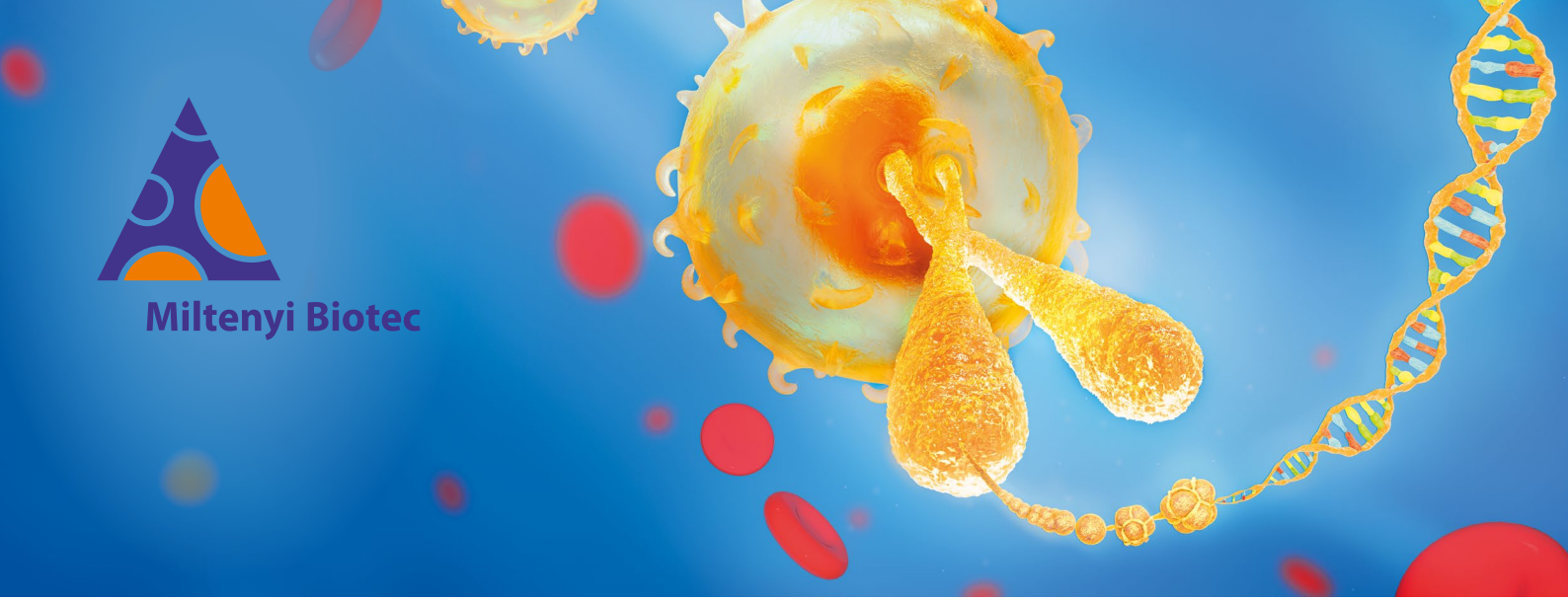

Isolation of CD19 CAR T cells directly from patient samples

Pure sample fraction for further downstream analysis

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Background

In recent years, chimeric antigen receptor (CAR) T cell therapy has been in the spotlight because of its high clinical relevance in the fight against certain hematological malignancies. The therapy is based on CARs, which are genetically engineered synthetic receptors expressed on immune cells to recognize and eliminate cells expressing a specific target antigen.[1] Patient-derived T cells (autologous) or T cells from healthy donors (allogenic) are used to generate CAR T cells. The procedure typically includes the following steps: After isolation of T cells out of blood, cells are genetically modified, followed by *ex vivo* expansion before reinfusion into the patient. Following reinfusion, the CAR binds specifically to a surface antigen and kills the cancer cell.

Despite the high rate of complete remissions of 57–93% of the CAR T cell products[2], the treatment has limitations. Some treatments are unsuccessful and lead to relapse or patients are resistant to CAR T cell therapy. The possible mechanisms can be very complex. However, to move the field forward, increased research efforts are needed to address the challenges and analyses of CAR T cells during treatment.[2]

Here we show how CAR T cells from low frequency material are isolated by the MACSprep™ CD19 CAR MicroBead kit, human to increase the sensitivity of further downstream applications. Using MACS® Technology, we were able to isolate CAR T cells directly from patients' whole blood samples to analyze fully functional CAR T cells (fig. 1). The analysis of the cells days after infusion or at different stages of therapy enables us to investigate CAR T cells in more detail and improve CAR T cell research.

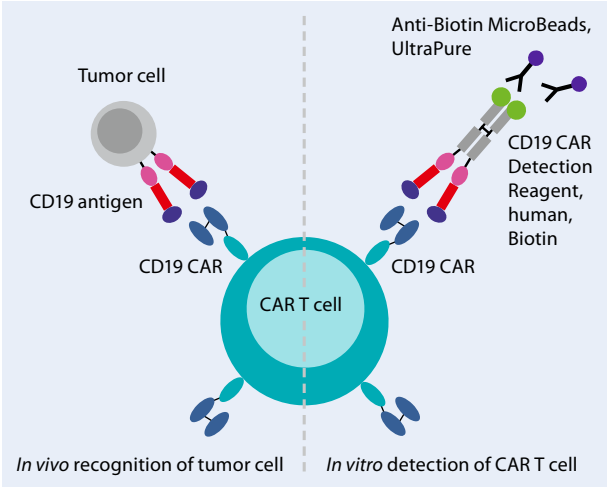

Figure 1: Schematic overview about CAR T cells *in vivo* and method of isolation *in vitro*. During treatment CD19 CAR T cell receptor recognizes a specific antigen to kill the tumor cell. For separation of CAR T cells, the CD19 CAR is detected by the CD19 CAR Detection Reagent, human Biotin. The addition of Anti-Biotin MicroBeads makes it possible to isolate CAR T cells directly from donors' blood.

Materials and methods

To isolate fully functional CAR T cells, high quality cell separation is required. For successful and efficient isolation of CD19 CAR T cells, we used the MACSprep CD19 CAR MicroBead Kit, human, which is based on MACS Technology. With this kit, we can directly isolate CAR T cells from patients' blood. Separation was performed following the manufacturer's instructions (fig. 2). A sample of 25 mL whole blood was collected at least 11 days after infusion of the CAR T cell products KYMRIAH® (tisagenlecleucel) or Yescarta™ (axicabtagene ciloleucel).

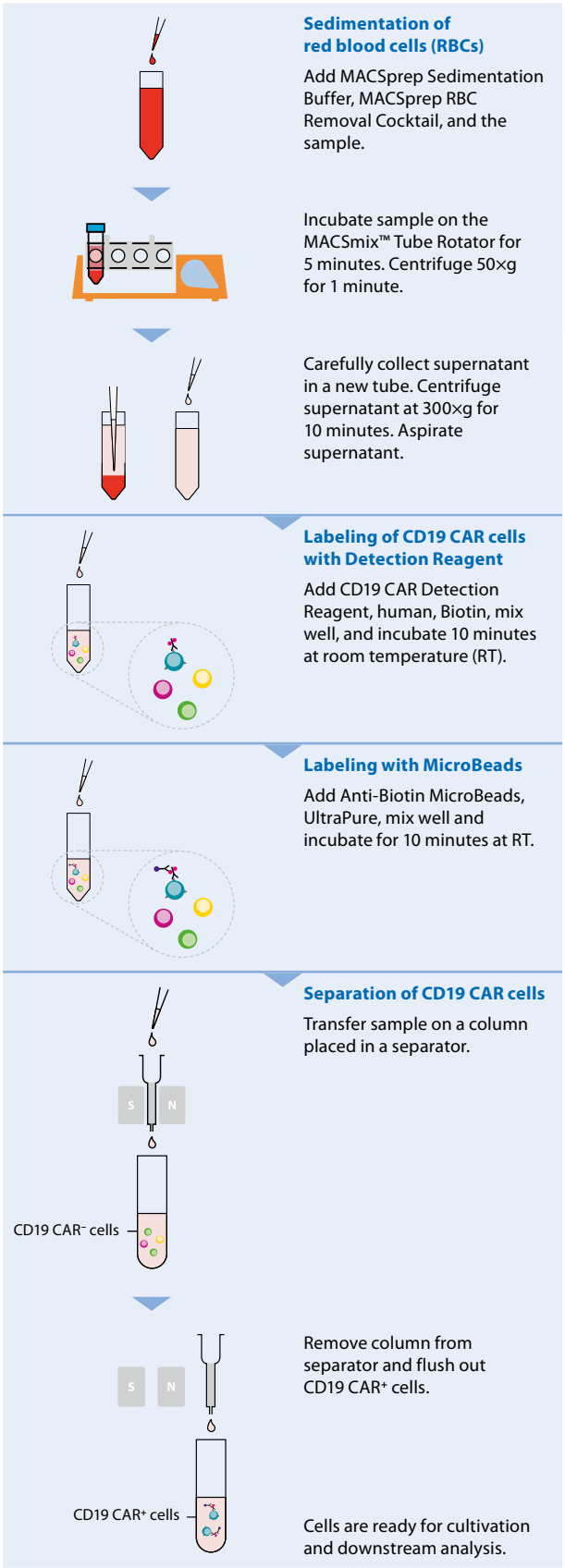

Figure 2: Protocol overview of isolation of CD19 CAR T cells from whole blood samples

Cell separation

Cell separation was performed directly from donors' blood by positive isolation using the MACSprep CD19 CAR MicroBead Kit, human. The advantage is that with direct cell separation from blood, the time-consuming and highly user-dependent density gradient centrifugation can be omitted. During the first isolation step red blood cells (RBCs) are aggregated and sedimented. After removal of RBCs, cells are labeled with CD19 CAR Detection Reagent, human, Biotin to detect CD19 CAR T cells. For magnetic separation, Anti-Biotin MicroBeads were used next, which bind to the Biotin label of the CD19 CAR Detection Reagent, human to separate the CD19 CAR T cells via magnetic separation.

Measurement of purity

For the measurement of purity after isolation, cells were fluorescently stained with CD19 CAR Detection Reagent, human, Biotin, Biotin Antibody-PE, CD45-VioGreen®, CD3-FITC (Table 1), and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals. The purity of CD19 CAR cells after separation is defined by the frequency of CD19 CAR T cells among CD3 cells in the positive fraction.

For staining	Clone	Order no.
CD45 Antibody, anti-human, VioGreen, REAfinity™	REA747	130-110-776
CD3 Antibody, anti-human, FITC, REAfinity	REA613	130-113-700
Biotin Antibody, PE, REAfinity	REA746	130-111-068
CD19 CAR Detection Reagent, human, Biotin		130-115-965

Table 1: Antibody panel used to analyze the purity of CAR T cells in the positive fraction.

Results

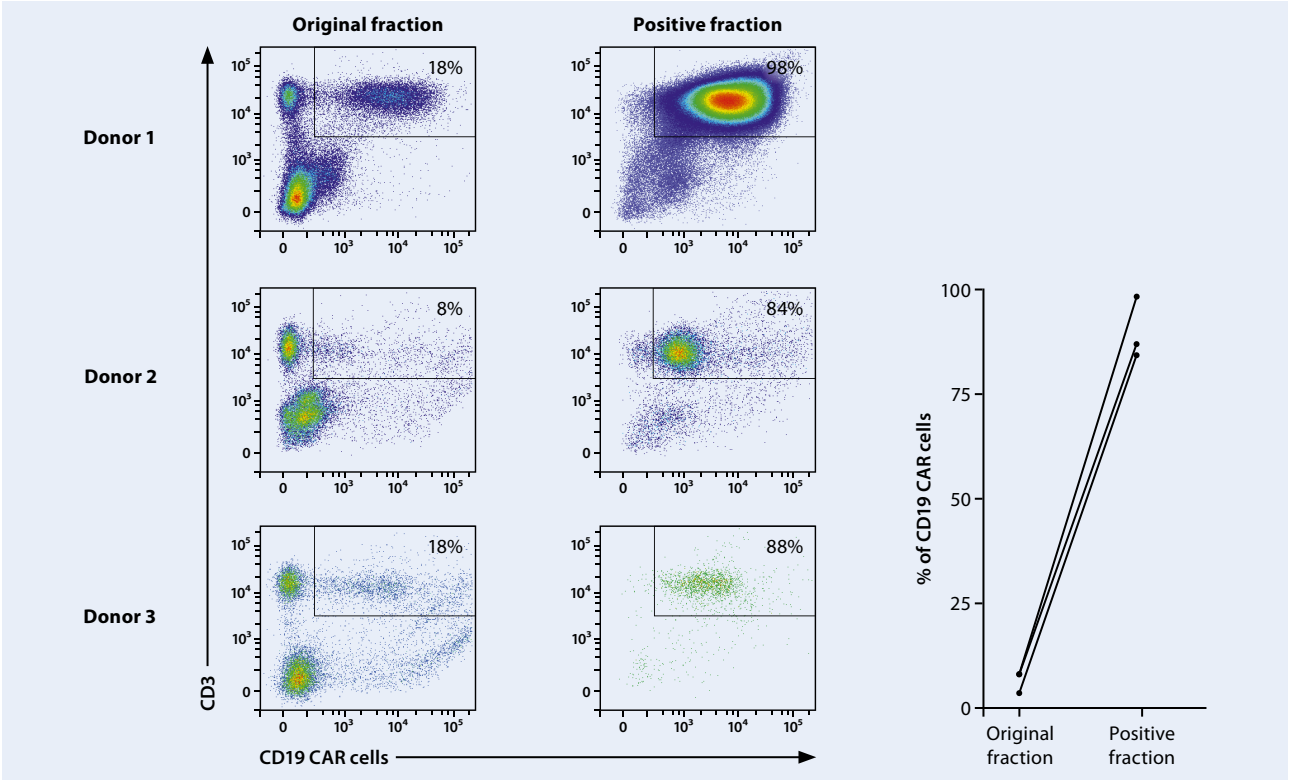

Figure 3: Isolation of CD19 CAR T cells from patient samples. Blood was collected at least 11 days after infusion with one of the commercial CAR T cell products KYMRIAH® or Yescarta™.

The purity of the isolated fraction is around 90 ± 7% (mean ± SD). The variation in purity of CD19 CAR T cells might be due to the differences in human samples, experimental setup, and starting frequency of CD19 T CAR cells. After separation, the isolated CD19 CAR T cells could be directly collected for further downstream analysis.

Conclusions

MACSprep CD19 CAR MicroBead Kit, human can isolate highly pure CD19 CAR T cells from patient blood to increase the sensitivity of the downstream assays. These data show that CAR T cells from the FDA-approved CAR T cell therapies can be isolated by using our MACSprep CD19 CAR MicroBead Kit. With an average purity of 90%, high purity was achieved. Separation of CAR T cells increases the sensitivity of further experiments to investigate CAR T cells during CAR T cell treatment.

Advantage to separate CD19 CAR T cells with magnetic separation:

- Increase sensitivity for downstream analysis, such as flow analysis, *in vitro* assays, and in-depth monitoring
- Enables the analysis of rare cells
- Fast and simple procedure
- No need for specially trained personnel, compared to cell sorting by flow cytometry
- Cells can be directly used for further downstream applications

References

1. Sterner, R. C. and Sterner, R. M. (2021) CAR-T cell therapy: current limitations and potential strategies. Blood Cancer J. 11(4):1-1
2. Xu, X., *et al.* (2021) Challenges and clinical strategies of CAR-T cell therapy for acute lymphoblastic leukaemia: overview and developments. Front immunol. 11:569117. doi: 10.3389/fimmu.2020.569117.

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