Materials and methods
CD14+ monocytes and naive CD4+ T cells were isolated from human peripheral blood mononuclear cells (PBMCs) using CD14+ MicroBeads, human, and Naive CD4+ T Cell Isolation Kit II, human, respectively. Monocytes were differentiated into immature Mo-DCs (imMo-DCs) during a 6-day culture in the presence of GM-CSF and IL-4. Maturation of imMo-DCs into mature Mo-DCs (mMo-DCs) was performed over the course of 24 hours in the presence of IL-1β, IL-6, TNF-α, and PGE₂. Allogenic cocultures of monocytes or differentiated Mo-DCs with freshly isolated naive CD4+ T cells were performed for 7 days.

Results
In vitro generated Mo-DCs assumed the typical DC phenotype (fig. 1). During differentiation, monocytes down-regulated the expression of CD14. In contrast, mMo-DCs expressed various DC markers that are involved in the formation of immunological synapses between DCs and naive T cells, including CD80, CD86, and MHC II (HLA-DR). Mature Mo-DCs also expressed DC activation markers CD83, CD40, and CCR7.

![Figure 1: Immunophenotyping of monocytes, imMo-DCs, and mMo-DCs. Cells were labeled with MACS® antibodies specific for the respective markers and analyzed with the MACSQuant® Analyzer. Black: isotype controls, red: marker-specific antibodies.](image-url)
Allogeneic naive CD45RA+CD45RO− T cells, labeled with cell-tracking dye, were cocultured with monocytes, imMo-DCs, or mMo-DCs (fig. 2A, B). After 7 days, cell numbers and the CellTrace™ Violet staining intensity of the T cells were analyzed (fig. 2C). In contrast to monocytes, both, imMo-DCs and mMo-DCs induced T cell proliferation as indicated by the increase in cell count and by the reduction of CellTrace™ Violet staining. T cell proliferation was highest when mMo-DCs were used as antigen-presenting cells (fig. 2D).

**Conclusion**

CD4+ T cells and CD14+ monocytes isolated with MACS® Isolation Kits and MicroBeads on e.g. the autoMACS® Pro Separator and cultured with MACS Cytokines can be successfully used for MLR assays.

For more data, download our poster

Monocyte-derived dendritic cells (Mo-DCs) generated with CliniMACS Prodigy® are functional and fulfill requirements for cancer vaccines at www.miltenyibiotec.com/Mo-DCs

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**Product**

<table>
<thead>
<tr>
<th>Order no.</th>
<th>Cell isolation</th>
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<tr>
<td>130-092-545</td>
<td>autoMACS® Pro Separator</td>
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<tr>
<td>130-094-131</td>
<td>Naive CD4+ T Cell Isolation Kit II, human</td>
</tr>
<tr>
<td>130-050-201</td>
<td>CD14 MicroBeads, human</td>
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</tbody>
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**MACS Cytokines for Mo-DC differentiation**

- Human GM-CSF, premium grade (50 µg) - Order no. 130-093-865
- Human IL-4, premium grade (25 µg) - Order no. 130-093-921

**MACS Cytokines for Mo-DC maturation**

- Human IL-1β, premium grade (25 µg) - Order no. 130-093-563
- Human IL-6, premium grade (25 µg) - Order no. 130-093-931
- Human TNF-α, premium grade (50 µg) - Order no. 130-094-023
- Human PGE₂, not available

**Flow cytometry**

- MACSQuant®, Analyzer 10 - Order no. 130-096-343

**MACS Antibodies**

Various order nos. please refer to www.miltenyibiotec.com/antibodies

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