Comparison of CD45⁺ depletion methods for enrichment of disseminated tumor cells in bone marrow samples

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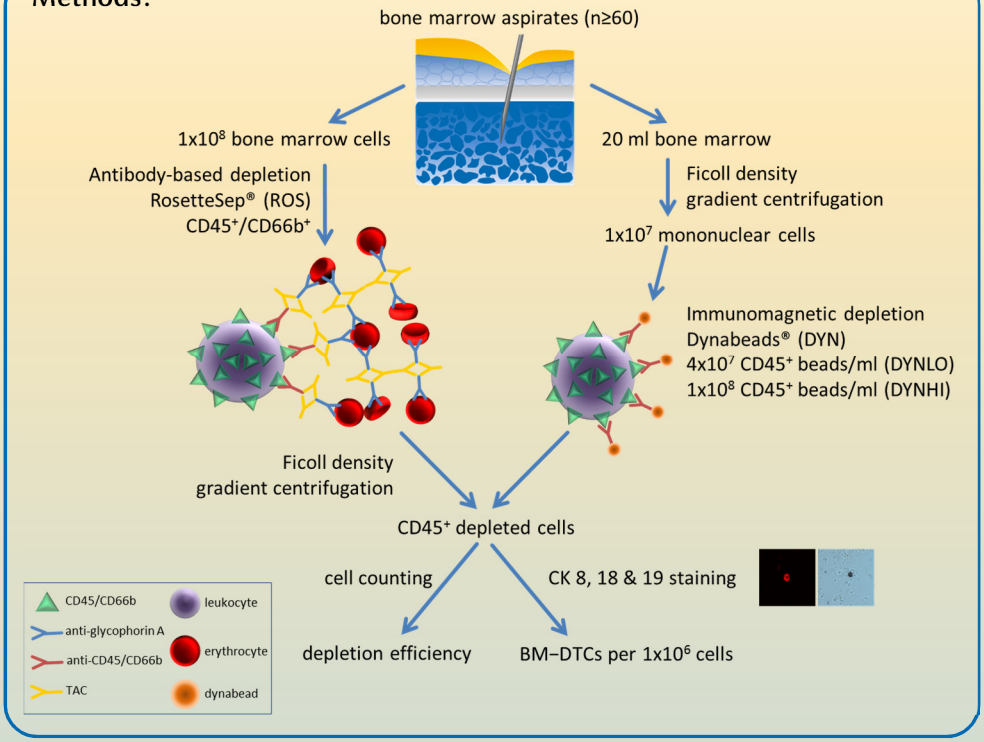
Background: Disseminated tumor cells (DTC) in bone marrow (BM) samples are, with a frequency of approx. one DTC per $1x10^6$ cells, extremely rare cells. The most common method to enrich DTCs is Ficoll density gradient centrifugation. In order to increase the efficiency of bone marrow-DTC enrichment we tested three different strategies to deplete CD45⁺ cells.

Methods:



Results:

- CD45⁺ cells could be depleted most efficiently up to 500-fold using ROS, followed by 44-fold with DYNHI and 20-fold using DYNLO (Fig.1A)
- DTCs could be detected in 32.7% (DYNLO), 12.5% (DYNHI) and 72.1% (ROS) of the patient samples
- The immunomagnetic depleted DYNLO samples (mean=0.48 per $1x10^6$ cells) had slightly higher DTC counts (8.33 vs 6.35 DTCs per $1x10^6$ cells) compared to the DYNHI samples (mean=0.39 per $1x10^6$ cells) (Fig.1B)
- DTC numbers were significantly higher (p<0.0001) in the ROS samples (mean=5.60 per $1x10^6$ cells) with up to 40 DTCs per $1x10^6$ cells (Fig.1B)

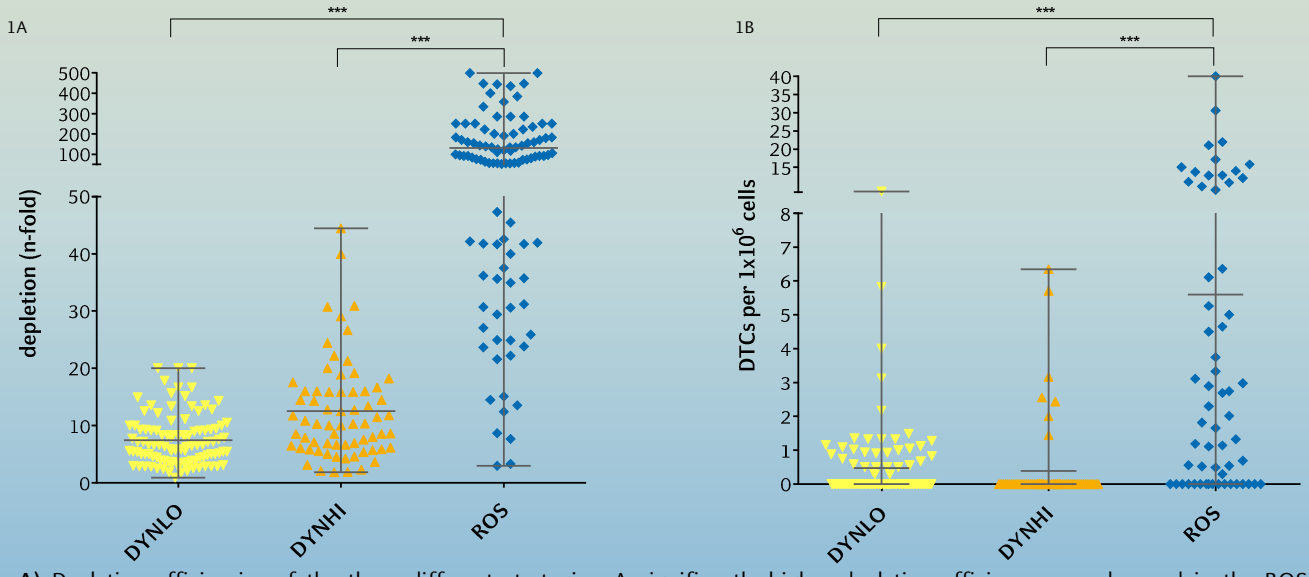


Figure 1: A) Depletion efficiencies of the three different strategies. A significantly higher depletion efficiency was observed in the ROS samples (p<0.0001, Mann-Whitney test) compared to the immunomagnetic depletion strategies DYNLO and DYNHI. **B)** Number of detected DTCs per one million cells in the depleted bone marrow samples. A significantly higher number of DTCs was observed in the ROS samples (p<0.0001, Mann-Whitney test) compared to the immunomagnetic depletion methods DYNLO and DYNHI.

Conclusions: In our hands the ROS procedure was most efficient in depleting CD45⁺ cells from bone marrow samples. This method enabled a high input of bone marrow material ($1x10^8$ mononuclear bone marrow cells) leading to an increased DTC detection rate in bone marrow samples from tumor patients.