

Computer processing of large datasets in the diagnosis of cancer micrometastases in the bone marrow

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Abstract. To detect disseminated tumor cells (DTC) in the bone marrow in patients with solid tumors by flow cytometry it is necessary to analyze a very large number of cells (up to 100 million myelokaryocytes). We have proposed the use of a new generation flow cytometers with acoustic focusing, making it possible to analyze up to 20 mln events in one file. To improve the accuracy of our analysis we used fluorochromes with non-overlapping emission spectra, which makes it possible to minimize the need of setting compensation of cytometer. Identification of the target cell population detected by sequential logic gating.

1. Introduction

During 2 years after the last ISMRC meeting in Paris several important and new aspects of that research took place. 1st is prove of Flow Cytometric detection of DTC. These rare cells can be identified by flow cytometry using acoustic focusing cytometers - Attune and later versions, these allows to collect large files (up to 100 million cells) with very high speed and good resolution. These aspects of research were discussed in details during 13th International Conference “Haematopoiesis Immunology”[1].

The aim of research work is to detect disseminated tumor cells (DTCs) in bone marrow of cancer patients using high speed flow cytometry with acoustic focusing.

2. Materials and methods

New-generation flow cytometers Attune have several unique advantages:

- Acoustic focusing
- High sensitivity
- High speed cell harvesting
- Ability to create large files
- Enables rapid detection of rare events, such as disseminated tumor cells (DTC)
- Minimal variation, even at high sample rates
- Less variability in results
- Data accuracy



We assessed possibility of DTC detection in morphologically normal bone marrow (BM). The study was made on 7 BM samples from patients with hematopoietic malignancies in clinical and hematological remission

DTC location area was determined in a model system with MCF-7 breast cancer cells added to 20–100 million normal BM cells (figure 1). The MCF-7 cancer cells were easily seen among myelokaryocytes and formed cell cluster with lack of common leukocyte antigen CD45 (figure 2).

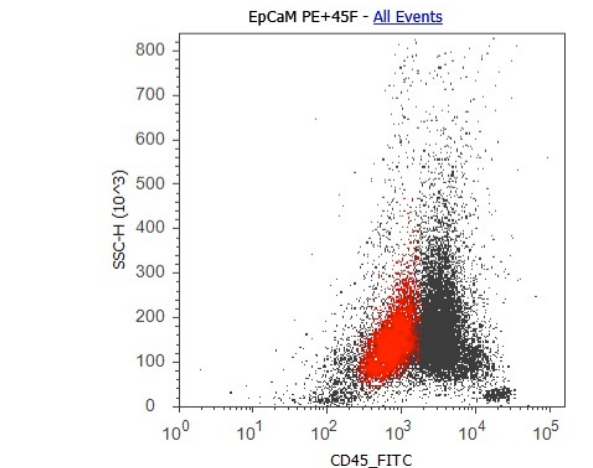


Figure 1. Red Gate – EpCam+CD45- in coordinates of side scetter (Y axis) and CD45 (X axis).

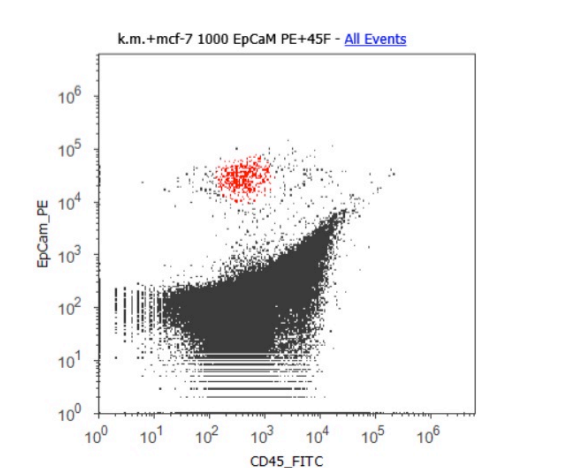


Figure 2. Red Gate – EpCam+CD45- in coordinates of EpCam (Y axis) and CD45 (X axis).

New generation of acoustic focusing instruments allows adjusting resolution to 107. As well, direct comparison of flow cytometric detection of DTCs without positive or negative selection and flow cytometric detection after immunomagnetic enrichment of DTCs showed that during enrichment meaningful DTCs loss may be seen (figure 3 and figure 4).

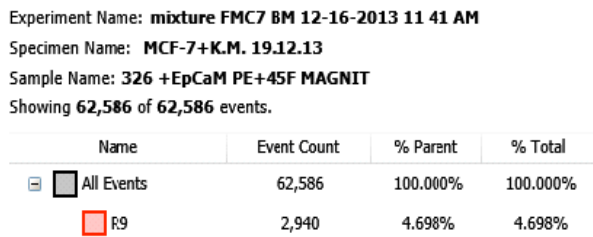


Figure 3. With immunomagnetic enrichment.

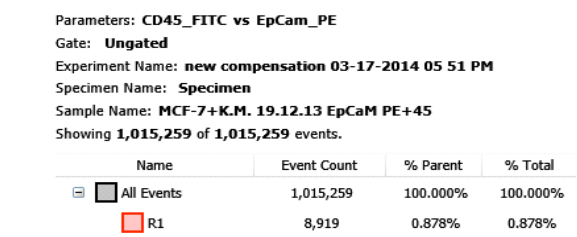


Figure 4. Without immunomagnetic enrichment.

We proved a possibility of creation large files up to 40 mln events and more. However the software let us view and analyse only 20 mln events. Collection of such a large file took 3 hours 20 minutes. According to the manual it is ten times faster (figure 5 and figure 6).

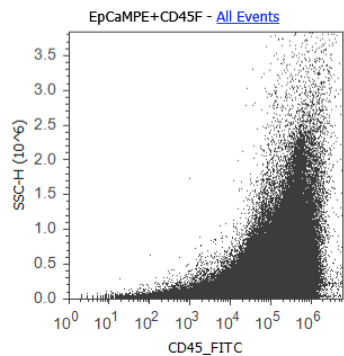


Figure 5. Example visualization of the generated file.

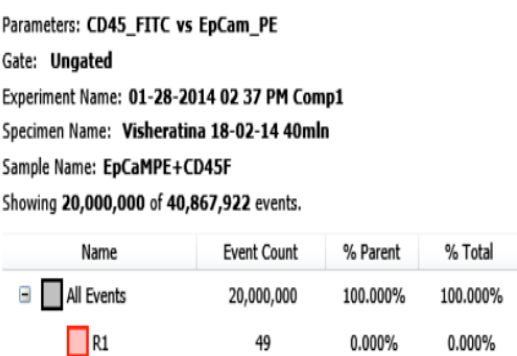


Figure 6. Statistics supporting 40 million events.

3. Clinical application

3.1. DTCs in gastric cancer patients

The presence of DTC in BM of patients with advanced gastric cancer is a poor prognostic factor associated with a high risk of early progression (figure 7 and figure 8). BM-DTC were identified significantly more frequently in cases with EGJ and total gastric cancer, and in patients free from gastric carcinomatosis; there was also a correlation with tumor histology (figure 9 and figure 10).

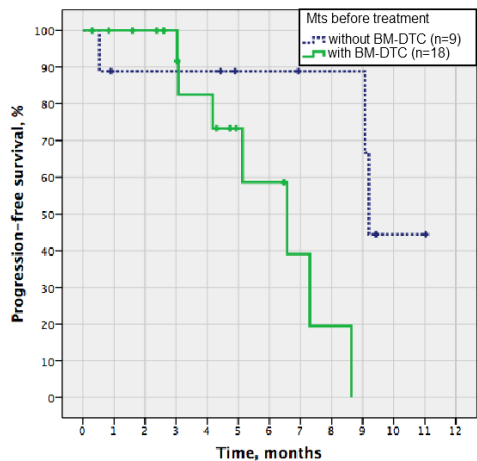


Figure 7. The comparison of progression-free survival between DTC-negative and DTC-positive patients.

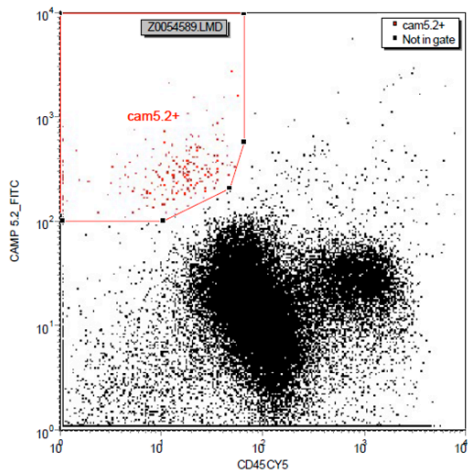


Figure 8. Clinical case of DTCs in bone marrow of gastric cancer patient.

3.2. DTCs in breast cancer patients

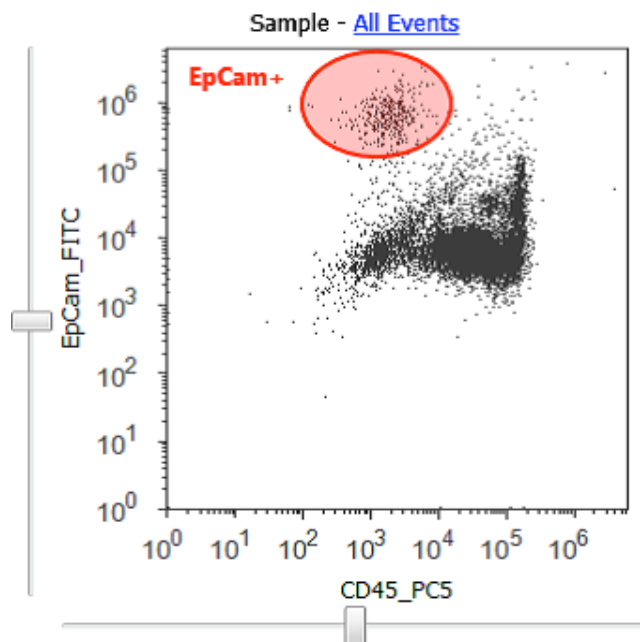


Figure 9. Clinical case of DTCs in bone marrow of breast cancer patient.

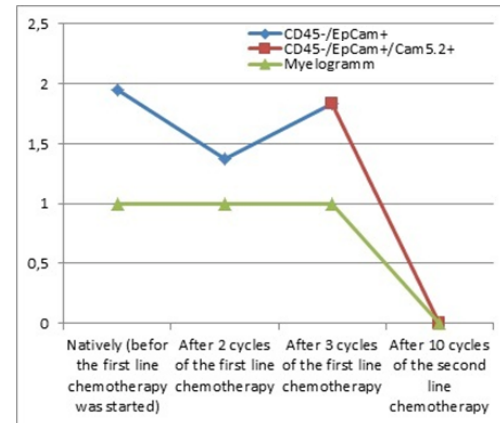


Figure 10. Monitoring of DTCs in bone marrow of breast cancer patient during chemotherapy.

4. Conclusion

Thus, DTCs detection has an important clinical value. We proved possibility of estimation of DTCs by flow cytometry, which has a number of advantages, such as a high speed of analysis and the ability to detect 1 cell per 10 mln myelocaryocytes. Due to the limitation of standard methods of diagnostics, quantification of DTCs could be used in an assessment of efficiency of system therapy by the dynamic of DTCs level.

References

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