Monitoring cytogenetic remission state of CML patients using automated scanning of hypermetaphase-FISH

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Introduction

The detection of the translocation (9;22)(q43;q11) or the BCR-ABL rearrangement respectively is necessary for the diagnosis of CML. Furthermore, it is of utmost importance to monitor and to quantify the presence of the BCR-ABL rearrangement throughout the course of the disease. In 1995 Seong et al [1]. presented the method of hypermetaphase-FISH (HM-FISH), which combines a modified preparation of metaphases with FISH, and introduced it to CML diagnostics. Due to the higher number of analyzable metaphases the sensitivity is increased compared to classical cytogenetics and also quantification becomes more exact. In addition, monitoring of Ph-negative, BCR-ABL-positive CML is possible. The method relies on a high number of metaphases, but searching for metaphases in fluorescence mode is time consuming and a strain on the eyes. Therefore, automation is highly desirable. We used the automatic scanning system Metafer-MSearch from MetaSystems for the unattended search for hypermetaphases. In a recent study on 350 CML patients we showed, that results of HM-FISH correlate very closely with results obtained by chromosome banding analysis, which is up to now the gold standard method to evaluate treatment response in CML [2]. Furthermore, due to the high number of evaluated metaphases HM-FISH was more sensitive to detect BCR-ABL positive metaphases compared to chromosome banding analysis. Therefore, hyper-metaphase-FISH is a suitable method for routine diagnostics in CML and can also be used in other hematological malignancies to detect residual disease.

Methods

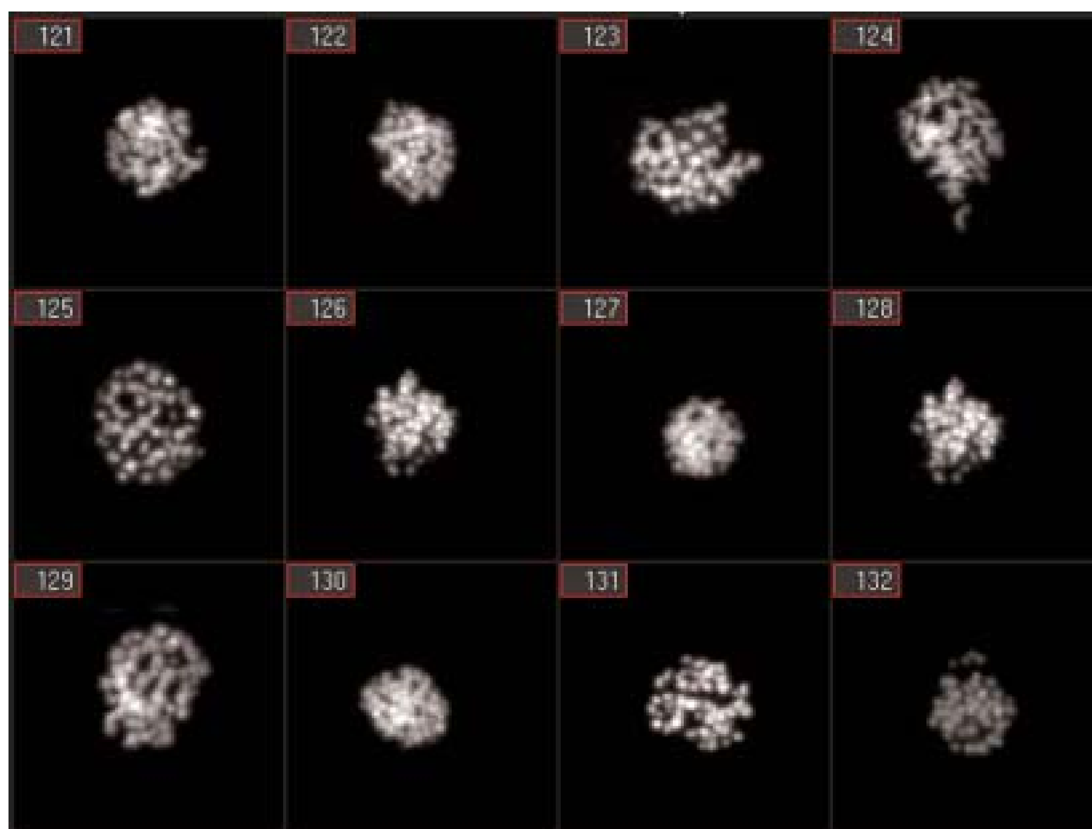
Hypermetaphase Preparation

For preparation of slides for HM-FISH bone marrow cells were cultured for 24h with medium supplemented with 20% fetal calf serum and a cytokine cocktail. Colcemid was added and cells cultured for another 24h followed by standard metaphase preparation. An area of 18 x 18 mm was hybridized using commercially available BCR-ABL probes. All available metaphases were evaluated for BCR-ABL- positivity.

Metaphase Scanning - General Set-up

The Metafer-MSearch slide scanning system is based on a motorized Axioplan microscope (Carl Zeiss, Germany) equipped with a motorized Maerzhaeuser 8-bay slide scanning stage. The microscope and the scanning stage are controlled by the Metafer software. During the scanning process the system tracks the best focus throughout the entire scan and moves the slide with reference to the fixed objective lens in a regular meander-like pattern. Each image field is analyzed, metaphases are identified and saved as gallery images (Figure 1). Once the scan has been completed, the gallery can be used to review the detected metaphases and false positives are deleted from the gallery. Subsequently, the metaphases are automatically relocated under the microscope and visually scored for fusion signals at higher magnification (Figure 2).

Figure 1. Image gallery of hypermetaphases



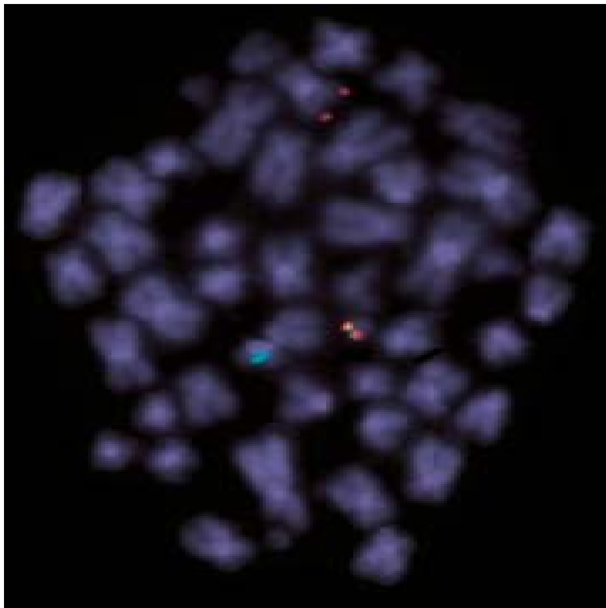


Figure 2. Representative hypermetaphase after hybridization with the BCR-ABL-specific probe. Two separate signals, one red one green are observed and an additional co-localisation signal composed of one red and one green spot, displaying the BCR-ABL-rearrangement, is present.

Results and Discussion

The automation of hypermetaphase analysis resulted in a tremendous improvement in throughput and accuracy. The unattended search in the fluorescent mode for hypermetaphases within the hybridized area was completed within 4 minutes. Generally, all hypermetaphases detected within the hybridized area were analyzed for BCR-ABL-fusion signals. The greater number of metaphases analyzed compared to manual scoring and conventional chromosome banding also improves the sensitivity and (statistical) accuracy of the results (*see below*). Another advantage over manual scoring lies in the proper documentation of each scan. Galleries with all object images, corresponding coordinates and measurement data are stored as individual files, allowing easy re-evaluation of results. As long as the microscopic slides are available, each cell can be easily relocated and re-examined.

In a study on 350 CML patients a median of 54 and a mean of 97 metaphases (range 1 to 521) were evaluated with HM-FISH. Chromosome analysis is still the gold standard for diagnosis and follow up studies in CML. However, especially in good responders the sensitivity of chromosome analysis is too low to detect residual disease. In 20% of cases that showed no Ph+ metaphases in chromosome banding analysis, HM-FISH was able to detect BCR-ABL+ metaphases. These data confirm the higher sensitivity of HM-FISH compared to chromosome banding analysis. Furthermore due to the higher number of analyzable metaphases with HM-FISH results become statistically more reliable. Whether the prognosis of patients with complete remission according to HM-FISH is significantly better than those with complete remission according to chromosome banding analysis but detectable BCR-ABL+ metaphases in HM-FISH has to be evaluated in clinical trials.

References

1. Seong DC, Kantarjian HM, Ro JY, Talpaz M, Xu J, Robinson JR, Deisseroth AB, Champlin RE, Siciliano MJ. Hypermetaphase fluorescence in situ hybridization for quantitative monitoring of Philadelphia chromosome-positive cells in patients with chronic myelogenous leukemia during treatment. *Blood* 1995; 86: 2343-2349.
2. Schoch C, Schnittger S, Bursch S, Gerstner D, Hochhaus A, Berger U, Hehlmann R, Hiddemann W, Haferlach T. Comparison of chromosome banding analysis, interphase- and hypermetaphase-FISH, qualitative and quantitative PCR for diagnosis and for follow-up in chronic myeloid leukemia: a study on 350 cases. Leukemia. 2002 Jan;16(1):53-9.
3. Schoch C, Haferlach T, Bursch S, Gerstner D, Schnittger S, Dugas M, Kern W, Löffler H, Hiddemann W. Loss of genetic material is more common than gain in acute myeloid leukemia with complex aberrant karyotype: A detailed analysis of 125 cases using conventional chromosome analysis and fluorescence in situ hybridization including 24-color-FISH. Genes, Chromosomes & Cancer 2002 in press.

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