

**A sensitive interphase FISH-assay for the early detection of Fanconi Anemia-specific MDS- and
AML-associated chromosomal imbalances on uncultivated bone marrow and peripheral blood
cells**

Holger Tönnies, Antje Gerlach, Reyk Richter, Eliska Volarikova, Stefanie Huber, Heidemarie Neitzel

Institute of Human Genetics, Charité, Universitätsmedizin Berlin, Berlin, Germany

Bone marrow (BM) failure in Fanconi Anemia (FA) patients followed by myelodysplastic syndrome or AML is strongly associated with the occurrence of FA-specific clonal chromosomal imbalances in BM cells. Our long term follow up data of FA patients with BM aspirations by conventional cytogenetics and comparative genomic hybridization revealed, that gains of 3q and losses of chromosome 7 are strongly associated with a poor prognosis and represent an adverse risk factor in FA for the progression into MDS/AML, but also for recurrent severe infections (Tönnies et al. 2003). We therefore established and validated a highly sensitive interphase-FISH (I-FISH) assay for the early detection of the most common adverse clonal chromosomal imbalances in uncultivated BM and peripheral blood (PB) cells from FA patients. However, the manual counting of up to 1000 interphase cells for each individual FISH-probe and target material (e.g. BM and PB direct preparations) by a human evaluator is time consuming and restricts the number of prospective I-FISH analyses which can be performed. Additionally, we know from our prospective clonality studies, that the adverse clones can appear and expand very fast. To permit more frequent high-throughput I-FISH analyses, we are now integrating an automated scanning system for unattended search and capturing of interphase nuclei in our analysis strategy. The multifunctional slide scanning system is based on a motorized fluorescence microscope equipped with a 8-bay slide scanning stage controlled by an adaptive scanning software (Metasystems). During the scanning process interphase cells and metaphase spreads are identified, pictures are saved, and I-FISH signals are counted automatically. Using this automated device and appropriate classifiers, a stable, evaluator-variation free detection and quantification of aberrant clones in BM and PB cells can be performed.

Much more data are necessary to correlate the cytogenetic findings with the clinical course of the disease. Therefore, we are now starting a pilot study with centers involved in the long term follow up of FA patients, in order to combine the relevant hematological data with the chromosomal findings after I-FISH. We ask primarily for non-cultured direct preparations of mononuclear cells from peripheral blood or/and from bone marrow probes and, if possible for DNA (the latter for confirmatory studies of aberrant I-FISH cases by CGH). The preparations for I-FISH scanning (simple direct fixation) can be made in parallel with normal chromosome preparations in any cytogenetic routine lab all over the world.

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