FLUORESCENCE IN SITU HYBRIDIZATION ANALYSIS OF ADULT AML ACCORDING TO RECENT UPDATES OF CLINICAL CYTOGENETICS GUIDELINES AND QUALITY ASSURANCE

Thesis

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List of Abbreviations

Abb.	*Full Term*
ABL	Abelson murine leukemia (viral oncogene homolog)
α-KG	Alpha ketoglutarate
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ANAE	Alpha naphthyl acetate esterase
Anti- RC84	Anti-human erythroleukemia cell line antigen
APL	Acute promyelocytic leukemia
AS203	Arsenic
ATRA	Alltrans retinoic acid
BAALC	Brain and acute leukemia cytoplasmic
BCR	B – Cell receptor
BM	Bone marrow
BMT	Bone marrow transplantation
Brdu	Bromodeoxyuridine
CAE	Chloroacetate esterase
CALGB	Cancer and leukemia group B
CAN	Candida gene
CBC	Complete blood count
CBF	Core – binding factor
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CEBPA	CCAAT/ enhancer binding protein A
CEP	Centromeric Probe
CGH	Comparative genomic hybridization

Abb.	*Full Term*
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CN-AML	Cytogenitically normal AML
CNS	Central nervous system
CR	Complete remission
CSF-1	Colony stimulating factor
DA	Distamycin A
DAPI	Diamino-phenyl indole
DEK	Dog embryo kidney
del	deletion
D-FISH	Double fusion Fluorescence insitu hybridization
D2HG	D2- hydroxyglutarate
DIC	Disseminated intravascular coagulopathy
DW	Distilled Water
ECOG	Eastern cooperative oncology group
EDTA	Ethylene diaminetetraacetic acid
EFS	Event free survival
ELN	European leukemia net
EM	Electron microscope
EVI1	Ectopic viral integration site 1
FAB	French-American-British
FBS	Fetal bovin serum
FCM	Flow cytometry
FITC	Fluorescein isothiocyanate
FLT3	FMS-like tyrosine kinase 3
G-banding	Giemsa banding
G-CSF	Granulocyte colony stimulating factor

Abb.	*Full Term*
GM-CSF	Granulocyte-monocyte colony stimulating factor
GVHD	Graft – versus – host disease
Hb	Haemoglobine
HCT-CI	Hematopoietic cell transplantation specific co-morbidity index
HER2	Human epidermal growth factor receptor 2
HLA-DR	Human leukocytic antigen
HMF	Hypermetaphase FISH
HSCT	Hematopoietic stem cell transplantation
IDH1	Isocitrate dehydrogenase 1
IFN-α	Interferon alpha
IL	Interleukin
Inv	Inversion
IPT	Immunophenotyping
IR	Incomplete remission
ISCN	International system for human cytogenetic numenclature
ITD	Internal tandem duplication
LDH	Lactate dehydrogenase
LSI	Locus specific identifier
LSP	Locus specific probe
MDS	Myelodysplastic syndrome
m-FISH	Multicolour- FISH
MLL	Mixed lineage leukemia
MN1	Meningioma 1 gene
MPN	Myeloprolifirative neoplasm
MPO	Myeloperoxidase

Abb.	*Full Term*
MRD	Minmal residual disease
m-RNA	Messenger RNA
NaF	Sodium fluoride
NCCN	National comprehensive cancer network
NEC	Non-erythroid compartment
NPM1	Nucleophosmin 1
NS	Non - significant
NSE	Non- specific esterase
OS	Overall survival
PAS	Periodic acid schiff
PB	Peripheral blood
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PCS	Phycoerythrin – cyanine 5
PE	Phycoerythrin
Ph	Philadelphia chromosome
PI3-Kinase	Phosphatidyl inositol 3-kinase
PML	Promyelocytic leukemia
RARA	Retinoic acid receptor alpha
Rb	Retinoblastoma gene
RE	Restriction enzyme
RFS	Relapse free survival
RPMI	Rosewell park memorial inistitute
RT-PCR	Reverse transcriptase polymerase chain reaction
S	Significant
SBB	Sudan black B
SBH	Southern Blot Hybridization

Abb.	*Full Term*
SCE	Sister chromatide exchange
SCFR (c-Kit)	Stem cell factor receptor
SCT	Stem cell transplantation
SF-FISH	Single fusion – Fluorescence insitu hybridization
SSC	Standard saline citrate
SWOG	Southwest oncology group
t	Translocation
TdT	Terminal deoxynucleotidyl transferase
TEM	Transmission electron microscopy
TKD	Tyrosine kinase domain
TKI	Tyrosine kinase inhibitor
TLC	Total leukocytic count
TNF	Tissue necrosing factor
UD	Undetermined
VEGF	Vascular endothelial growth factor
WCP	Whole chromosome painting probe
WHO	World health organization
WT1	Wilms tumor 1
-	Monosomy
+	Trisomy

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PROFESSIONAL GUIDELINES FOR CLINICAL CYTOGENETICS OF ACUTE MYELOID LEUKAEMIA

I-INTRODUCTION

Professional guidelines for Cytogenetics laboratories incorporate the standards imposed by regulatory bodies (Clinical Pathology Accreditation (CPA)) and by statute (Clinical Governance) **(Association for Clinical Cytogenetics, 2012).**

II-BACKGROUND

Cytogenetic analysis is integral to the diagnosis and/or classification of both AML and MDS, and is recommended in clinical guidelines **(Milligan et al., 2006 and Dohner et al., 2010)**. Detection of disease-specific abnormalities can allow the correct diagnosis to be reached, provide information on prognosis and allow a risk classification to be made. It can also be useful in assessing disease progression or response to treatment. This information can be used by the clinician to counsel the patient and to select the appropriate mode of treatment.

A) AML Clonal karyotype:

Abnormalities are found in leukaemic cells in approximately 55% of adult AML cases and 78% of childhood AML **(Milligan et al., 2006 and von Neuhoff et al., 2010)**, detection of these abnormalities can aid the diagnosis by demonstration of clonality .Many abnormalities are not specific

to AML and general markers of myeloid neoplasia are common. Within the World Health Organisation Classification of Tumours of Haematopoietic & Lymphoid Tissues 2008 **(Swerdlow et al., 2008)**, a number of diagnoses within AML are now defined by specific cytogenetic abnormalities.

B) Cultures:

It is recommended that a method of cell counting is used, so that final culture densities can be optimised to approximately 1x106 /ml. When there is sufficient sample, at least two culture types should be initiated to increase the chance of obtaining analysable abnormal metaphase preparations and detecting the abnormal clone. Standard one and/or two day cultures are appropriate for all myeloid disorders. It has been reported that growth factor supplements, such as granulocyte colony stimulating factor, granulocyte macrophage colony stimulating factor and interleukin-3, may improve the quality of cytogenetic preparations and facilitate chromosome analysis in myeloid malignancies **(Earle et al., 2007)**. Occasionally, AML may present as a solid, extra-medullary granulocytic sarcoma either with or without concurrent bone marrow infiltration. Cytogenetically, t(8;21)(q22;q22) or inv(16)(p13q22) are frequent findings. Tissue samples should be disaggregated to produce a cell suspension; this may then be cultured, processed and analysed in the same way as bone marrow.

C)Analysis:

1- G-banded analysis for AML and MDS at diagnosis:

For all diagnostic cases, if no clonal abnormality is detected by G-banding, a minimum of 20 metaphases must be

examined, with at least 10 of these being fully analysed. If fewer than 20 suitable metaphases are available, the result must be reported with a 'partial analysis' caveat. If 10 cells cannot be fully analysed in a case with normal cytogenetics then this must be considered a failed analysis. Where an abnormal clone is detected, examination of 10 metaphases is recommended, with at least five of these being fully analysed. Abnormal results may, if necessary, be reported on fewer metaphases, provided that the ISCN criteria for a clone are fulfilled (i.e. two or more cells with same extra chromosomes or structural rearrangements, or three or more with the same chromosome loss). FISH may be useful to corroborate the presence of abnormalities tentatively identified in such restricted analyses. In the case of finding a single cell with a potentially significant abnormality, such as loss of chromosome 7, it is recommended that a minimum of 10 additional cells are scored in order to exclude this, or that FISH analysis is considered.

2-FISH/RT-PCR analyses at diagnosis:

a)Acute promyelocyticleukaemia(APL): If APL is suspected at presentation, rapid analysis of RARA rearrangement status may be necessary and this may be best achieved by FISH or RT- PCR. It is important to distinguish between t(15;17)(q24;q21) PML-RARA and t(11;17)(q23;q21) ZBTB16(PLZF)-RARA in APL since these have differing responses to ATRA **(Sanz et al., 2009)**. In the situation where metaphase chromosomes are unavailable and FISH indicates RARA rearrangement but not PML-RARA fusion, then analysis should be undertaken to rule out ZBTB16 (PLZF) involvement. Since there are currently no commercial FISH probes for

ZBTB16(PLZF)-RARA, this may require referral to another laboratory.

It is important to note that variant rearrangements can occur which are both chromosomally cryptic and also normal by FISH analysis using both PML-RARA dual fusion and RARA break apart probes. RT-PCR analysis must be performed in such cases; this may require referral to another laboratory. NB/ the recently redefined location for PML is 15q24; this should be used in the translocation ISCN.

b)Inv(16)(p13q22): This subtle rearrangement may be overlooked in poor quality preparations. In cases where G-banding shows apparently normal chromosomes 16, FISH or RT-PCR analyses must be carried out if (i) the haematologist reports bone marrow morphology consistent with inv (16), or (ii) secondary abnormalities associated with inv(16), such as deletion of 9q or trisomy 22, are identified in the karyotype. Even if an inv (16) is detected by G-banding, it is recommended that FISH or RT-PCR be used to confirm the interpretation. bi-phenotypicleukaemia / leukaemia in infants.

3-Unsuccessful Karyotype – FISH panels :

When chromosome analysis is unsuccessful due to lack of metaphases or poor quality preparations, interphase FISH and/or RT-PCR should be considered to investigate abnormalities of established prognostic importance. The need for such studies will vary from case to case, taking into account the potential impact on clinical management approaches.

D) Interpretation and Reporting :

Abnormal results must be linked where possible to WHO classification subtypes **(Swerdlow et al., 2008)**. If a patient is known to be entered onto a particular trial, then the appropriate risk stratification should be given according to the trial classification. If there is a discrepancy with more recent data, then this should be highlighted.

1- Karyotype complexity :

Historically, the criteria used to classify karyotype complexity have not been clearly or consistently defined. The number of abnormalities used to define complexity differs in AML and MDS, and so reports must refer to a particular prognostic scheme; this can be problematic since a diagnosis is often not confirmed at the time of requesting cytogenetics, it is recommended to use the classification of complexity described in Grimwade et al. 2010 **(Grimwade et al., 2010)** which stipulates ≥ 4 abnormalities. The classification of Chun et al. 2009 **(Chun et al., 2009)** is recommended for scoring the number of cytogenetic abnormalities in MDS.

2-Prognosis :

Many cytogenetic abnormalities in AML carry a prognostic association. Information regarding prognosis may be derived from WHO **(Swerdlow et al., 2008)**, or where a specific prognosis is based on multiple publications or inclusion on national or international trials. In such cases, prognostic associations must be reported.