

STEM CELL AND LEUCOCYTE BIOLOGY

Title: Study of cytogenetic, immunophenotypic and biologic characteristics of AML with study of FLT3 gene mutations.

Year of commencement : 2003

Year of completion : 2006

Objectives:

1. Study FLT3 mutations and expression of multi-drug resistance protein, P-glycoprotein (P-gp) in patients of Acute Myeloid Leukemia (AML) to identify the high-risk population.
2. Study correlation between FLT3 gene mutations, expression of P-gp and immunophenotypic and cytogenetic abnormalities in patients of AML

Total 189 patients with diagnosed de novo acute myeloid leukemia attending the haematology department, K.E.M. Hospital during year 2004-2006 were included in this study. Diagnosis was based on the peripheral blood and bone marrow examination for morphology, cytochemistry and immunophenotypic studies. All clinical details including presenting features, treatment given and response to treatment were recorded.

Immunophenotyping :

A multi-parameter analysis based on Triple colour immunophenotypic study of the surface antigens were performed using combinations of Phycoerythrine (PE), Fluorescein Isothiocyanate (FITC) and PerCP conjugated monoclonal antibodies. The following antibodies were used

Myeloid markers: CD11c, CD13, CD14, CD 15, CD 33 and MPO

Lymphoid markers: CD2, CD3, CD4, CD5, CD 7, CD8, CD10, CD19, CD20, CD22,
CD23 and CD79a

Non lineage markers: CD34, CD45, and HLA-DR

Cytogenetics studies:

Cytogenetic analysis was performed using standard Giemsa banding techniques to stain metaphases cells obtained from unstimulated and after short-term cultures. The definition of a cytogenetic clone and descriptions of karyotype will follow the International System for Human Cytogenetic nomenclature. FISH analysis of certain samples was carried out using different specific probes.

FLT3 mutation studies:

Genomic DNA was extracted using a salting out procedure from fresh bone marrow or peripheral blood cells after Ficoll separation of mononuclear cells of all these 189 cases. Exon 11 and 12 of the FLT3 gene was amplified by genomic polymerase chain reaction. The amplified product was screened for mutation by running on 2% agarose gel and then resolved on 12% polyacrylamide gels. Abnormal band of the amplified fragment was excised, purified and will be sent for sequencing.

P-gp expression studies:

P-gp expression was studied by Flowcytometry using fluorescent label monoclonal antibodies to P-gp protein.

Results:

Table 1: Age and sex distribution of AML patients.

Age	Males	Females	Total
Children(1mon-12 yrs)	15 (57.7%)	11 (42.3%)	26
Adults (12-75 yrs)	89 (54.6%)	74 (45.4%)	163
Total	104 (55.0%)	85 (45.0%)	189

Table 2: Hematological profile of AML patients.

	Hb (gm%)	TC ($\times 10^9 / L$)	Platelet count ($\times 10^9 / L$)
Children	6.3 (2.5-12.4)	30 (1.3-417)	95 (10-542)

Adults	7.4 (2.2-15.4)	39 (0.6-370)	93 (2-3600)
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Table 3: FAB classification of AML patients:

	AML M0/M1	AML -M2	AML -M3	AML -M4	AML - M5	AML - M6	AML -M7
Children	7 (26.9%)	5 (19.2%)	6 (23.1%)	7 (26.9%)	-	-	1(3.8%)
Adults	28(17.2%)	37(22.7%)	35(21.5%)	33 (20.2%)	16(9.8%)	11(6.7%)	3(1.8%)
Total	35(18.5%)	42(22.2%)	41(21.7%)	40 (21.2%)	16(8.5%)	11(5.8%)	4(2.1%)

Immunophenotypic characteristics:

Table 4: Aberrant expression of lymphoid markers in AML patients

Markers	Number of patients
CD 2	6 (3.2%)
CD 3	2 (1.1%)
CD 7	14 (7.4%)
CD 10	2 (1.1%)
CD 19	21 (11.1%)
CD 34	56 (29.6%)
CD 56	15 (7.9%)
CD 7 + CD 34	20 (10.6%)

P-gp expression studies:

50 (37.3%) of 134 patients studied for expression of P-gp were found to be positive for P-gp expression. Of these patients, 6 were children and the remaining 44 were adults.

Cytogenetic studies:

Table 5: Chromosomal abnormalities of 189 AML patients.

Sr. No	Type of chromosomal abnormality	Number of patients
1	Hyperdiploidy (>50 chromosomes)	2 (1.1%)
2	Hypodiploidy	8 (4.2%)
3	Hypodiploidy / del (9) (q12-qter) / +12	1 (0.5%)
4	45,XX, t(1;X)(p21;q31), -7	1 (0.5%)
5	45,XY, -7	2 (1.1%)
6	45,XY, der(X), t(8;21;X), (q22;q22;p22.1)	1 (0.5%)
7	45,XY, del(3), - 7	1 (0.5%)
8	45,X, t(8;21), (q22;q22), -Y	2 (1.1%)
9	45,XX, t(8;21), (q22;q22), del(9), (q12-ter)	1 (0.5%)
10	46,XY, del(1)	1 (0.5%)
11	46,XY, del(7), (q32-ter)	1 (0.5%)
12	46,XY, del(7), (q21.1-qter)	1 (0.5%)
13	46,XY, del(9) (q12-qter)	2 (1.1%)
14	46,XX, del(16q), (q22 -ter)	1 (0.5%)
15	46,XY, t(9;22)(q34;q11)	2 (1.1%)
16	46,XX, t(6;9)(p23;q34)	1 (0.5%)
17	46,XX, t(8;21)(q22;q22), t(7;13)	1 (0.5%)
18	46,XY, t(8;21)(q21;q21)	3 (1.6%)
19	46,XX, +10	1 (0.5%)
20	46,XY, inv(16)(p12q24)	2 (1.1%)
21	46,XY, der(6), der(11)	1 (0.5%)
22	47,XX, +4	1 (0.5%)
23	47,XX, +10	1 (0.5%)
24	47,XX, +mar	1 (0.5%)
25	47,XY, +4	1 (0.5%)
26	47,XY, +8	3 (1.6%)
27	47,XX, +14	1 (0.5%)

28	47,XY, t(9;22) +21	1 (0.5%)
29	48,XX, +8, +10, del(13q), der(7q), der(9q)	1 (0.5%)
30	PML/RARA fusion	19 (10.1%)
31	PML-RARA negative	2 (1.1%)
32	Normal karyotype	74 (39.1%)
33	Karyotype not done	48 (25.4%)
	Total	189

FLT3 Mutation studies:

Of the total number of patients, 174 have been screened so far for FLT3-ITD mutation. Of these, 24 patients (13.8%) were positive for FLT3-ITD mutation.

Table 6: Age and Sex distribution of patients with FLT3 mutation:

Age	Males	Females	Total
Children(1mon-12 yrs)	1 (4.0%)	-	1
Adults (12-75 yrs)	16 (64.0%)	8 (32.0%)	24
Total	17 (68.0%)	8 (32.0%)	25

Table 7: Hematological profile of AML patients positive for FLT3 mutation:

Diagnosis	Hb (gm%)	TC (x109 / L)	Platelet count (x109 / L)
APML with FLT3 mutation	7.8 (4.2-13.0)	67 (1.0-260)	35 (10-120)
APML without FLT3 mutation	6.9 (4.1-12.4)	27 (0.6-210)	58 (8-175)
AML excluding APML with FLT3 mutation	7.3 (3.9-14.0)	89 (2.2-260)	255 (10-360)

AML excluding APLM without FLT3 mutation	7.1 (3.6-15.4)	59 (0.6-417)	107 (8-542)
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Table 8: FAB classification of AML patients positive for FLT3 mutation:

	AML -M0	AML M0/M1	AML -M2	AML -M3	AML -M4	AML - M5	AML - M6	AML -M7
Children	-	-	-	1 (4.0%)	-	-	-	-
Adults	1 (4.0%)	2 (8.0%)	7 (28.0%)	6 (24.0%)	4 (16.0%)	2 (8.0%)	1 (4.0%)	-
Total	1 (4.0%)	2 (8.0%)	7 (28.0%)	7 (28.0%)	4 (16.0%)	2 (8.0%)	1 (4.0%)	-

Table 9: Immunophenotypic characteristics in AML patients positive for FLT3 mutation:

CD Markers	CD2	CD4	CD19	CD56	CD34	pgp
APML	1 (14.3%)	-	-	-	-	1 (16.6%)
AML excluding APML	1 (5.9%)	1 (5.9%)	3 (17.6%)	3 (17.6%)	6 (35.3%)	5 (29.4%)

Correlation with cytogenetics:

Of the 25 patients positive for FLT3-ITD, 5 (20%) patients showed PML/RARA fusion. Of the remaining 20 patients, 16 (64%) patients showed normal cytogenetics and the remaining 4 (16%) showed the following cytogenetic abnormalities:

45,XY, der(X), t(8;21;X), (q22;q22;p22.1)

Hypodiploidy / del (9), (q12-qter), +12

46,XY, del(7), (q32 -ter)

46,XY, t(9;22) (q34;q11)

Fig 1: FLT3 Internal Tandem Duplication (ITD)



