Impact of FLT3-ITD diversity on response to induction chemotherapy in patients with acute myeloid leukemia

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SUPPLEMENT

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PATIENTS AND METHODS

Patient characteristics, treatment, molecular diagnostics and ethical standards

All AML patients included in this retrospective analysis were diagnosed and treated at the Department of Internal Medicine II (Haematology, Oncology and stem cell transplantation) at Universitätsklinikum Jena, Jena, Germany. Analysis of FLT3-ITD was part of central diagnostic procedures of the *Ostdeutsche Studiengruppe für Hämatologie und Onkologie* (OSHO). At primary diagnosis, all patients gave their informed consent for FLT3 mutation screening using genomic DNA of bone marrow or peripheral blood samples.

All patients received intensive induction chemotherapy according to one of the following protocols of the OSHO: AML96 or AML2002 protocol containing idarubicine for patients up to 60 years old and AML97 or AML2004 protocol containing mitoxantrone for elderly patients. Patients with acute promyelocytic leukemia were excluded from this study.

Clinical characteristics of all investigated AML patients are demonstrated in Table S1 and Table S2.

All procedures were in accordance with ethical standards of the institutional research committee and with the Declaration of Helsinki. The study has been approved by the Jena University Hospital Ethics Committee (4871-07/16). Written informed consent was obtained from all patient included in this study.

Response criteria

The haematological response to chemotherapy was assessed as follows: complete remission (CR) was defined as less than 5% of bone marrow blasts with normal peripheral counts (neutrophils > 1.000/μl and platelets > 100.000/μl) including a normal differential haemogram while patients with CRp had platelets below 100.000/μl. Partial remission (PR) was defined as

blasts between 5% and 20% following chemotherapy while bone marrow blasts of 20% and more were considered as refractory AML.[1]

Cytogenetic analyses of AML samples

Karyotype was regularly determined at primary diagnosis of AML according to standard methods and is provided in all investigated patients. Leukemic blasts isolated from samples of bone marrow or peripheral blood were karyotyped according to the International System for Human Cytogenetic Nomenclature.[2] Risk classification based on cytogenetic analysis was performed according to the recommendations published by the European Leukemia Network.[3]

Cloning and analysis of FLT3-ITD fragments

DNA was extracted from bone marrow aspirates or peripheral blood. Genomic DNA was isolated using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Sequence analysis was performed as previously described.[4] Cloning of the FLT3-ITD PCR fragments was performed using the TOPO pCRII TOPO®TA cloning Kit according to the manufacturer's instructions (Invitrogen, Groningen, Netherlands). Transformed bacteria were plated on LB Ampicillin agar plates and selected for insertions by color screening. White colonies were picked and propagated by overnight culture. Plasmid DNA was extracted by the alkaline lysis method using the solutions provided by Qiagen Plasmid Buffer Set (Qiagen, Hilden, Germany).

In order to identify the type and extent of duplication the isolated plasmid DNA was subjected to automatic sequencing at Seqlab Sequence Laboratories GmbH (Göttingen, Germany).

Bioinformatic analysis was done using Snapgene software. Mutant sequences were analyzed by alignment to the wildtype sequence of FLT3 gene (NCBI Reference Sequence: NG_007066.1).

Statistics

For statistical analyses the SPSS software package, version 22 (SPSS, Chicago, IL) was used. Event times were described using Kaplan Meier curve. Chi-squared test and multivariate logistic regression analysis for CR and log rank test for LFS were applied to evaluate significance between subgroups.

TABLES

Table S1: Clinical characteristics of AML patients with single FLT3-ITD

	n = 43
Sex (male/ female)	17 (39.5%) / 26 (60.5%)
Median age at diagnosis, years (IQR)	57 (47 - 63)
Cytogenetic risk group favourable intermediate unfavourable	 1 (2.3%) 37 (86.1%) 5 (11.6%)
AML history *de novo* AML antecedent MDS	 36 (83.7%) 7 (16.3%)
Remission after induction chemotherapy CR PR BP	 31 (72.1%) 7 (16.3%) 5 (11.6%)
FLT3-ITD allelic ratio N-terminal (median) C-terminal (median)	 0.60 (0.11 -1.0) 0.61 (0.09 – 1.0)
LFS, median months (95% CI)	11.0 (5.4 – 16.6)
OS, median months (95% CI)	56.0 (33.3 – 78.6)
Allogeneic stem cell transplantation	28 (65.1%)

Abbreviations: IQR, interquartile range; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CR, complete remission; PR, partial remission; BP, blast persistence; LFS, leukemia-free survival; OS, overall survival (median LFS and OS are based on Kaplan-Meier curves); CI, confidence interval

Table S2: Clinical characteristics of patients with allogeneic stem cell transplantation

	n = 28
Median age at diagnosis, years (IQR)	52 (43 - 59)
Remission prior to transplantation	
CR1	21 (75.0%)
CR2	1 (3.6%)
PR	3 (10.7%)
Relapse / refractory disease	3 (10.7%)
Transplantat	
MRD	5 (17.8%)
MUD	15 (53.6%)
mMUD	8 (28.6%)
Conditioning regimen	
myeloablative	11 (29.3%)
RIC	17 (60.7%)
GvHD	
no or grade I	16 (57.1)
grade II - IV	12 (42.9)
LFS, median months (95% CI)	16.0 (0.5 – 31.5)
OS, median months (95% CI)	47.0 (31.1 – 62.9)

Abbreviations: CR1, first complete remission; CR2, second complete remission; PR, partial remission; MRD, matched related donor; MUD, matched unrelated donor; mMUD, mismatched unrelated donor; RIC, reduced intensity condition; GvHD, graft-versus-host disease; LFS, leukemia-free survival; OS, overall survival (median LFS and OS are based on Kaplan-Meier estimates); CI, confidence interval

FIGURES

FIGURE S1: Amino acid sequences of all FLT3-ITDs with highlighting of tyrosine, serine and threonine residues: analysis of amino acid composition of all FLT3-ITD sequences (n = 58). Tyrosine (yellow), serine (green) and threonine (blue) residues are highlighted.

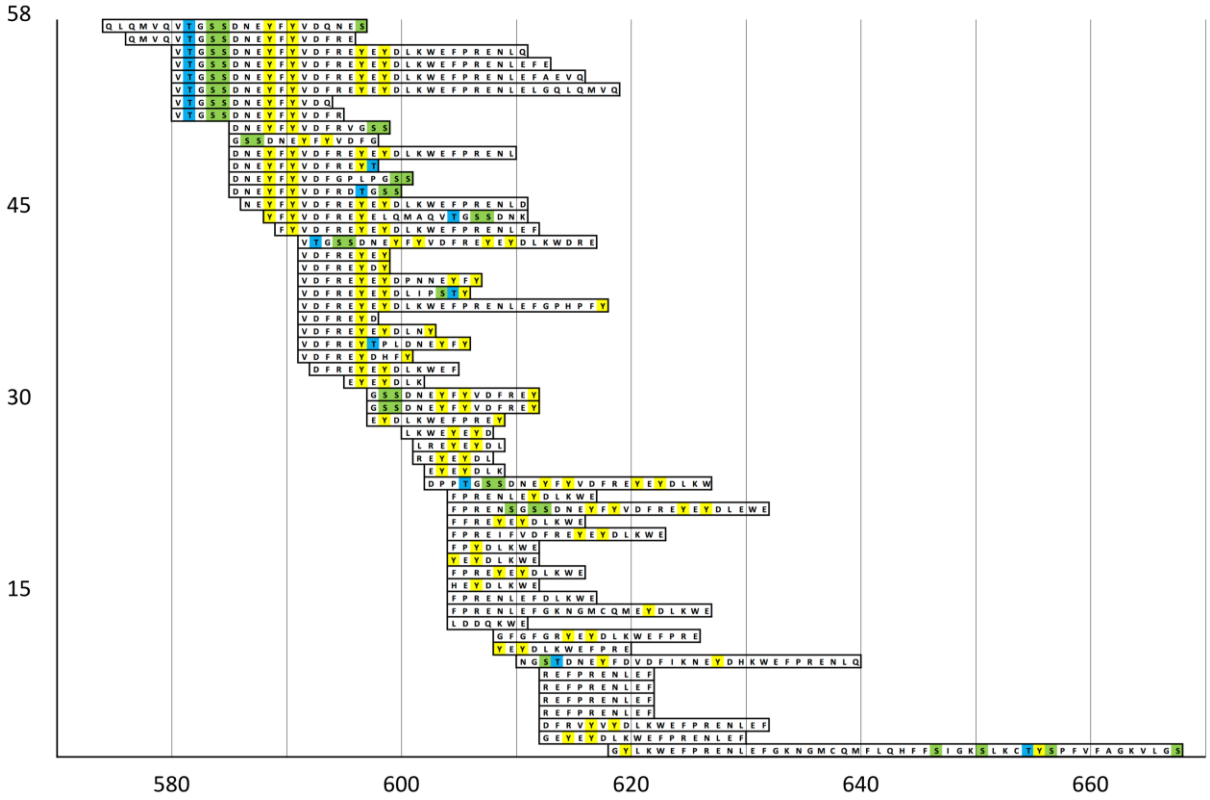
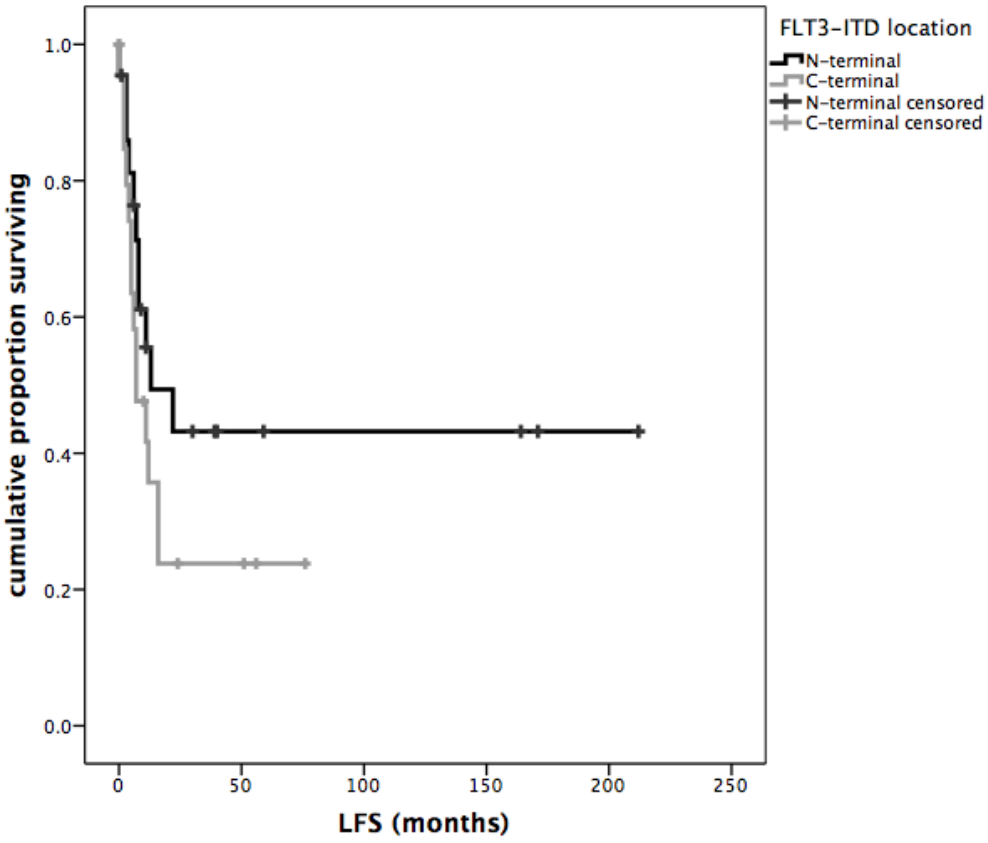


Figure S2: Kaplan-Meier curve of leukemia-free survival (LFS) dependent on localization of FLT3-ITD (N-terminal vs. C-terminal): LFS does not differ significantly (median LFS 13 month (95% CI: 0 - 33.1 months) vs. 7 months (95% CI: 0.2 - 13.8 months, *P* = 0.188).



REFERENCES

1. Swerdlow S, Campo E, Harris NL, Jaffe E, Pileri S, Stein H. WHO classification of Tumours of Haematopoietic and Lymphoid Tissues (International Agency for Research on Cancer, Lyon, France). 2008.
2. Brothman AR, Persons DL, Shaffer LG. Nomenclature evolution: Changes in the ISCN from the 2005 to the 2009 edition. Cytogenet Genome Res. 2009;127(1):1-4.
3. Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010;115(3):453-474.
4. Scholl S, Loncarevic IF, Krause C, Kunert C, Clement JH, Hoffken K. Minimal residual disease based on patient specific Flt3-ITD and -ITT mutations in acute myeloid leukemia. Leuk Res. 2005;29(7):849-853.