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CHARACTERISTICS AND OUTCOME OF THERAPY-RELATED MYELOID
NEOPLASMS: REPORT FROM THE ITALIAN NETWORK
ON SECONDARY LEUKEMIAS

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Abstract

Therapy-related myeloid neoplasms (t-MN) are a complication of cytotoxic treatment for primary tumors and autoimmune diseases.

We report data on 277 t-MN patients, recruited between 1999 and 2013 by the Italian Network on Secondary Leukemias (104 retrospectively and 173 prospectively registered). Median age at t-MN diagnosis was 64 years (range 21-87). Most frequent primary malignancies (PM) were lymphoproliferative diseases and breast cancer. One hundred-thirty-three patients had received chemotherapy (CHT), 43 patients radiotherapy (RT) and 101 patients combined CHT/RT for PM. Median time between cytotoxic treatment and t-MN was 5.7 years, with t-MN following RT alone associated with significantly longer latency, compared to CHT or combined CHT/RT (mean 11.2 vs 7.1 years, $p=0.0005$). Addition of Topoisomerase-II inhibitors to alkylating agents was associated with shorter latency compared to alkylating agents alone (median 6 vs 8.4 years, $p=0.02$).

Median survival was 14.6 months from t-MN diagnosis, and was significantly longer in patients treated with allogeneic stem cell transplantation. Significant factors for survival at the multivariable analysis included age, adverse karyotype and degree of anemia.

Our data underline the prognostic importance of karyotype and age in t-MN, similar to *de novo* AML. Treatment approaches should not preclude the use of conventional treatments for younger t-MN patients, including allogeneic stem cell transplantation as potentially curative approach.

INTRODUCTION

Therapy-related myeloid neoplasms (t-MN) include acute myeloid leukemias (t-AML) and myelodysplastic syndromes (t-MDS) occurring in patients treated with radiotherapy and/or chemotherapy for cancer or autoimmune diseases. t-MN may arise from few months to several years after the primary tumor, are associated with clinical and biologic unfavorable prognostic features and have been recognized as a distinct entity by the 2008 World Health Organization (WHO).¹

Latency between primary malignancy and t-MN depends on age at diagnosis of the primary malignancy, type of cytotoxic treatment, cumulative dose and dose-intensity.²⁻³ Since less than 5% of patients exposed to cytotoxic treatment develop a t-MN, individual susceptibility has also been suggested.^{2,4-9} From a biological point of view, t-MN are characterized by high frequency of chromosomal abnormalities, complex karyotypes and TP53 mutations.¹⁰ Other somatic mutations including those involving epigenetic and spliceosome machinery enzymes, are rare, opposite to *de novo* AML and MDS.^{8,11}

t-MN account for about 10-15% of all AML and are characterized by poor survival. At present, survival data have been mostly retrospectively collected, with limited clinical information available.^{5,12-13} In 2009, we initiated a multicenter epidemiological registry using a Web-database, with the purpose of collecting characteristics and outcome of t-MN, observed at Italian Hematology or Oncology Divisions. The registry included a retrospective and a prospective data collection. In this paper, we report on 277 t-MN patients, diagnosed with a t-MN between 1999 and 2013.

METHODS

Between May 2009 and September 2013 a total of 325 adult t-MN patients consecutively observed at 22 Hematology and Oncology Centers were systematically registered in the web-database, whose access was restricted to selected users and was password-protected. Data of all patients with t-MN diagnosed between January 1999 and April 2009 and recorded at the same Centers were also retrospectively included in the web-database. The study had been approved by the Ethical Committees of all participating Centers and patients gave informed consent to data collection and analysis.

t-MN diagnosis was made locally according WHO classification criteria. Of 332 secondary leukemia patients included in the web-database, 277 [117 males and 160 females; median age 64 years (range 21-87 years)] were *bona-fide* t-MN, arising after chemo- or radiotherapy for a primary malignancy or after immunosuppressive therapy for autoimmune diseases. In 34 cases (10.5%), leukemia represented a second cancer in patients treated for the primary malignancy with surgery alone. In 14 patients, the primary disease was a myeloproliferative neoplasm (myelofibrosis, 7; polycythemia vera, 3; essential thrombocythemia, 3; hyperosinophilic syndrome, 1) treated with hydroxyurea. These patients were excluded from the subsequent analysis since leukemic evolution is recognized as part of natural history of myeloproliferative neoplasms. Only 7 therapy-related acute lymphoblastic leukemia (t-ALL) were registered in the data-base during the time period covered by the registry. This paper will focus on the 277 t-MN patients, defined according to the WHO classification.¹

One hundred-four patients were retrospectively (from 1999 to April 2009) and 173 patients prospectively (from May 2009 to September 2013) registered. Clinical t-MN data were collected, together with details concerning the primary treatment, including chemotherapy and/or radiotherapy. Cytotoxic agents to treat the primary disease were classified according

to the mechanism of action, and were grouped into alkylating agents, topoisomerase 2 inhibitors, antimetabolites and antitubulin drugs.

Latency interval was defined for all patients as the time between the “first” cytotoxic therapy and the first bone marrow sampling diagnostic for t-MN, also in 19 patients with a history of 2 or more previous cancers. Patients were followed up until death or through September 2013. Cytogenetic risk groups were defined according to the European Leukemia Net (ELN)¹⁴ [*favorable*: t(8;21)(q22;q22), inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); *intermediate*: normal karyotype, t(9;11)(p22;q23), cytogenetic abnormalities not classified as favorable or adverse; *adverse*: inv(3)(q21q26.2) or t(3;3)(q21;q26.2), t(6;9)(p23;q34), t(v;11)(v;q23), -5 or del(5q), -7, abn(17p) and complex karyotype]. Therapy-related acute promyelocytic leukemia (APL, N= 16 patients) was diagnosed according to standard criteria and was included in the favorable group. Molecular genetic data were not available.

Statistical analysis

t-MN characteristics were analyzed for retrospectively (n=104) and prospectively (n=173) collected patients. Differences in the distribution of prognostic factors in patient subgroups were analyzed using the X^2 or Fisher's exact test and the Wilcoxon test.

Overall survival (OS) was defined as the time from t-MN diagnosis to death or date of last follow-up. OS was calculated using Kaplan–Meier estimate, while differences in survival were calculated using the log-rank test in univariate analysis and the Cox regression model in multivariate analysis. All significant factors in univariate analysis and all clinical important variables were used to perform multivariate analysis.

Since the treatment could represent a confounding variable in the multivariate analysis for survival, this analysis was stratified for treatment type, including best supportive care, standard chemotherapy, hypomethylating agents, autologous and allogeneic stem cell

transplantation as stratification factors. Final models were also evaluated with backward, forward and stepwise function. In all, 95% confidence intervals (CI) were reported for the main summary statistics. Statistical analyses were performed using SAS and the statistical software environment R (<http://www.r-project.org/>). HR were calculated for failure (death).

All comparisons are two-sided with a nominal significance level of 5% ($p \leq 0.05$).

RESULTS

Patients' Characteristics

Clinical characteristics of 277 adult t-MN patients, divided in the retrospective and prospective cohorts are shown in Table 1. According to morphology, there were 157 AML and 120 MDS. The primary disease (PD) was a hematological neoplasm in 111 patients (43%), a solid tumor in 155 (53%), and an autoimmune disease in 11 patients (4%).

Nineteen patients (6.5%) had a history of two or more previous cancers treated with cytotoxic therapy for at least one PD, in some cases one of previous cancers had been treated with surgery alone. No data on cancer familiarity was available, but at least in some of these patients inherited cancer predispositions may be suspected. Median age at PD diagnosis was 52 years (range 21-82 years).

Among hematological malignancies, the most frequent were lymphoproliferative neoplasms (70 Non-Hodgkin and 18 Hodgkin lymphomas, 6 chronic lymphocytic leukemias, 12 multiple myeloma). In addition, there were 1 acute lymphoblastic leukemia (ALL), 2 AML and 2 APL.

The 5 t-MN following acute leukemia were considered therapy-related since karyotype was different from that of the primary acute leukemia. In particular, 2 cases with a previous APL developed a non-APL t-AML with complex karyotype; 1 patient developed t-AML after ALL; 2 patients, one with a previous t(8;21) AML and the other with a previous inv(16) AML presented a deletion of 7q in t-MN. Median latency between PD and t-MN in these cases was 3.7 years (range 2.6-7.5 years).

Breast cancer (69/155 cases, 44.5%) was the most frequent primary solid tumor, other sites were the genitourinary system (14 prostate, 5 bladder, 1 kidney, 8 uterus and 5 ovarian cancers), followed by colon-rectum (18 patients); lungs (9); thyroid (8); and others (central nervous system, 6; oropharynx, 4; sarcoma, 3; stomach; 2; skin, 2; unknown, 1).

One hundred-thirty-three patients had received as primary treatment chemotherapy (CHT), 43 patients radiotherapy (RT), and 101 patients a combined CHT/RT treatment. Patients with a previous autoimmune disease had received immunosuppressive therapy, including antimetabolites and topoisomerase II inhibitors in 6 and 5 cases respectively.

Median latency between cytotoxic treatment and t-MN was 5.7 years (range 0.5-48 years). t-MN following RT alone had a significantly longer latency than following chemotherapy or combined CHT/RT regimens [11.2 ± 1.8 vs 7.1 ± 0.4 years, $p=0.0005$, mean \pm standard error of the mean (SEM)(Figure 1)]. Details on the chemotherapy drugs received were available for 200 patients, detailed dose of the drugs used was not available. Patients received alkylating agents alone ($n=25$), alkylating agents combined to topoisomerase II inhibitors \pm other drugs ($n=89$) or to antimetabolites \pm anti-tubulin drugs ($n=39$), topoisomerase inhibitors ($n=21$), antimetabolites ($n=18$), or other combinations ($n=8$). Addition of topoisomerase II inhibitors to alkylating agents was associated with shorter latency (mean 6 ± 0.5 vs 8.4 ± 1.1 years, $p=0.02$, mean \pm SEM, figure 1). No other significant differences in latency times emerged.

According to morphology, there were 157 AML [median bone marrow blasts 45% (range 20-99)] and 120 t-MDS [median bone marrow blasts 3% (range 0-18)]. In t-MDS, median hemoglobin (Hb) concentration was 8.9 g/dl (range 5.4-14.6), median white blood cell count (WBC) $3.84 \times 10^9/L$ (range 0.8-25), and median platelet count $71 \times 10^9/L$ (range 7-1164). In t-AML, median Hb was 8.5 g/dl (range 4-12.3), median WBC $21.5 \times 10^9/L$ (range 0.5-112) and median platelet count $53 \times 10^9/L$ (range 6-220).

Karyotype was available in 212 patients and was abnormal in 66% of patients (Table 1). There were 23 patients with favorable (11%), 106 with intermediate (50%) and 83 with unfavorable karyotype (39%). No differences in karyotype distribution emerged according to primary disease treatment ($p=0.4$). Involvement of the MLL gene was identified in only 4 patients, who had received topoisomerase II inhibitors alone in 1 case or in combination with an alkylating agent in 3 cases.

The distribution of patients was similar in the retrospective and prospective cohorts, considering age at t-MN diagnosis, type of primary disease and of t-MN, treatment of primary disease and median latency between primary cytotoxic therapy and t-MN onset. There was a trend towards a higher frequency of complex karyotypes and a lower rate of balanced translocations in the prospective cohort ($p=0.05$).

The median follow-up was obviously significantly longer for the retrospective cohort, compared to the prospective cohort (29.8 months vs 12.9 months, $p=0.0001$, table 1).

Survival and Prognostic Factors

Data on t-MN treatment was available for 244 patients. Standard induction therapy (with 7+3 regimens or including triple agents) was administered to 83 patients (34%), 50 (20.5%) received hypomethylating treatment (azacitidine, VIDAZA, Celgene™), 42 (17%) allogeneic and 11 (4.5%) autologous stem cell transplantation, and 58 best supportive care (24%).

Median overall survival (OS) was 14.6 months (Figure 2A). Allogeneic stem cell transplantation was associated with the longest survival, compared to patients receiving other treatment types [median OS: 58.8 months for allogeneic stem cell transplantation (SCT) versus 12.1 months, $p<0.0001$, Figure 2B]. Although a lower number of elderly patients underwent allogeneic stem cell transplantation versus younger patients (14 vs 28 respectively), it is however interesting to underline that no significant differences in overall

survival emerged comparing younger (below the age of 60 years) and elderly patients ($p=0.6$).

The survival analysis adjusted for recruitment (retrospective vs prospective) identified age at t-MN diagnosis, platelet counts below $30 \times 10^9/L$, hemoglobin level, adverse karyotype and allogeneic stem cell transplantation compared to best supportive care as significant prognostic factors for OS (Table 2, Figure 2C). Other factors, including bone marrow blast percentage (below or above 20%), previous chemo- or radiotherapy were not significantly associated with survival. Interestingly no survival differences were observed comparing t-MDS to t-AML.

The multivariable analysis stratified for treatment type identified age, hemoglobin as continuous variable, and unfavorable karyotype as independent prognostic factors (Table 3). Accordingly, we developed a clinical prognostic score attributing one point to age over 60 years and one point to unfavorable karyotype. This resulted into three prognostic groups including patients with 0, 1 or 2 adverse factors. The score was established in the retrospectively collected patient series, defined as “Training cohort”, and included 73 patients with available complete data set. Patients with 0, 1 or 2 negative features had a significantly different overall survival (median OS 58.9 months, versus 16.1 and 10.7 months, respectively, $p=0.003$) (Figure 3A). The score was then validated in the “Validation cohort”, including 116 patients prospectively registered. Patients with score 0 (20 patients, median OS not reached), had a significantly better survival when compared to patients with 1 or 2 poor risk features ($n=56$ and $n=40$ patients, OS: 14 and 8.9 months, respectively, $p<0.0001$) (Figure 3B).

DISCUSSION

Here, we report on a large series of t-MN patients registered by the Italian network on secondary leukemias. The present study is hospital-based and does not necessarily reflect t-MN/t-AML population trends, it is however in line with other epidemiological studies, confirming breast cancer and lymphoproliferative diseases as the most frequent primary solid and hematological malignancies.^{3,5,12-13,15} Similar to previous reports, overrepresentation of breast cancer among solid tumours became even more evident when the analysis was limited to females, where breast cancer accounts for 70%.³ Recently, in a population-based survey of 426,068 adults treated with chemotherapy for cancer, Morton et al⁵ identified 801 t-AML cases, which translates into a 4.7-fold increased risk for AML, compared to the general population. Over three decades, there has been an increase of t-AML risk following treatment of non-Hodgkin lymphoma, a decline of the risk for ovarian cancer, myeloma and possibly lung cancer. This study also reported emerging t-MN risk-groups, coincident with the expanding use of chemotherapy for cancers of the esophagus, anus, cervix, endometrium, and prostate. Emerging cancer types account for 16% of cases in our registry.

Lymphoproliferative diseases were the most frequent primary disease, accounting for 32% of cases. Hodgkin lymphoma was the primary tumour in 6% of patients, at a frequency slightly lower than that reported by the AMLSG group (10%) and the Chicago series (25%).¹²⁻¹³ This probably reflects the evolution of HL treatment over time, variable approaches in different countries, with the recent tendency to avoid nitrogen mustards, reduce the number of chemotherapy cycles and the extension of radiotherapy fields.¹⁶⁻¹⁷ A retrospective analysis, reported 106 t-MN cases in 11952 HL patients (0.9%) treated within the German Hodgkin Study Group between 1993 and 2009, and identified four or more cycles of escalated-BEACOPP as significant t-MN risk factor.¹⁸

Median t-MN latency in our study was 5.6 years, without any significant difference between t-MN arising after hematological malignancies versus solid tumors. Recently, age at primary disease and prior treatment with mitotic inhibitors have been identified as independent prognostic factors for short latency in t-MN.³ We found that patients treated with radiotherapy alone had a significantly longer latency to t-MN compared to chemotherapy-including regimens. Very long latency times question whether these AML and MDS should be considered as “second” tumours in cancer-susceptible patients, rather than true t-MN.¹⁹⁻²⁰

In our population, cytogenetic abnormalities were very frequent, with single or multiple abnormalities in 64% of evaluable cases. Karyotype was unfavorable in 39% of patients, and involved chromosomes 5 or 7 deletions in most cases. We found no differences in the distribution of karyotype abnormalities according to treatment, probably due to the fact that most of patients received combinations of different drugs.

Median survival of our population was 14.6 months from t-MN diagnosis. Survival analysis in t-MN is hampered by the absence of prospective studies and the usual exclusion of t-MN from clinical trials. Our registry, although patients were not homogeneously treated, reflects two different time periods (between 1999 and April 2009, for the retrospective group and between May 2009 and September 2013 for the prospective cohort). In the multivariate analysis, we identified age at t-MN diagnosis, hemoglobin level, and adverse karyotype as independent prognostic factors for survival. These factors, together with an antecedent hematologic or autoimmune disease and platelet counts below $30 \times 10^9/L$, forecasted inferior survival in a recent predictive model for survival developed by Ornstein et al³ in 58 t-AML patients. In our patients, no survival differences were observed grouping patients according to primary disease. On the other hand, age over 60 and adverse karyotype, which are classical prognostic parameters also in *de novo* AML, could be included in a

simplified prognostic score, which identified three patients groups with significantly different survival probabilities. This score may prove useful in the clinical setting and may be improved by future inclusion of somatic mutations in the model.

In addition to classical parameters, as age and karyotype, somatic mutations have been shown to play a major role as prognostic determinants for *de novo* AML, where mutations of FLT3, NPM1 and CEBPA have been shown to improve survival prediction¹⁴. In t-MN, FLT3, NPM1, epigenetic and spliceosome mutations have been shown to occur in a minority of patients^{8,12}, indicating that in these diseases karyotype and the high frequency of TP53 mutations¹⁰ have a dominant pathogenetic and prognostic role. Further studies using whole exome sequencing technologies may help to identify possible novel recurrent somatic mutations in t-MN.

The heterogeneous treatments of our t-MN, ranging from best supportive care to intensive chemotherapy, hypomethylating agents and stem cell transplantation, does not allow definite conclusions on the best treatment choice in t-MN, mostly typical of elderly patients. Allogeneic stem cell transplantation, performed in only 17% of cases was the most successful treatment option, and elderly fit patients suitable for allogeneic stem cell transplantation had an overall survival similar to younger patients. In this line, a previous multicenter study showed that t-AML patients with good performance status, enrolled in conventional GIMEMA trials, had treatment response rates similar to *de novo* AML.²¹ This paper showed that t-AML was an adverse prognostic factor for death in complete remission and overall survival, but not relapse, in younger intensively treated patients, probably reflecting cumulative toxicity of primary disease and leukemia treatment.²

Altogether, these data suggest that t-AML patients in good performance status should be recruited in conventional trials. Allogeneic stem cell transplantation should be considered

for unfavorable karyotypes, including monosomal karyotype, which has been shown to play an important prognostic role (^{20,22-26}). In the future, additional factors should be considered, in particular mutations of TP53, which identify patients with poor survival despite SCT ²⁷. In this setting, other factors associated with poor outcome included older age and depth of anemia.

In conclusion, our registry confirms that breast cancer and lymphoma are the predominant diseases at risk for the development of t-MN. In t-MN, individual treatment decisions should be taken independently from a history of previous cancer, but taking into account cumulative toxicity due to antecedent treatment. t-MN “per se” should not be considered as prohibitive factor for standard therapy, since some t-MN are not biologically unfavourable, and there is a fair proportion of favourable/intermediate karyotype. Younger t-MN patients should be enrolled in front-line conventional chemotherapy trials for acute myeloid leukemia or MDS, including allogeneic stem cell transplantation, which could be taken in account also for elderly fit patients.

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Conflict of interest

Authors did not declare any conflicts of interest with the work presented in this paper.

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Figure Legends

Figure 1.

t-MN Latency according to treatment of the primary disease

Data on specific drugs used to treat the primary disease were available for 200 patients. t-MN following RT alone had a significantly longer latency than following chemotherapy or combined CHT/RT regimens (11.2 ± 1.8 vs 7.1 ± 0.4 years, $p=0.0005$). Furthermore, addition of topoisomerase II inhibitors to alkylating agents was associated with shorter latency (6 ± 0.5 vs 8.4 ± 1.1 years, $p=0.02$).

Figure 2

Overall Survival

Median overall survival for the entire patient cohort was 14.6 months (A). Allogeneic stem cell transplantation (Allo-SCT) was associated with significantly longer survival than autologous SCT (Auto-SCT), standard induction chemotherapy (CHT), hypomethylating treatment with azacitidine (AZA), or best supportive care (BSC) (B). Unfavourable karyotype, defined according to ELN risk groups¹³ was significantly associated with poor survival (C).

Figure 3. Overall survival in t-MN patients stratified according to the clinical prognostic score.

A) In 73 patients retrospectively registered and with available data (defined as “Training set”), the multivariable analysis identified adverse karyotype according to ELN¹³ and age over 60 as independent prognostic factors for survival. Patients with 0, 1 or 2 negative features had a significantly different overall survival (median OS 58.9 months, versus 16.1 and 10.7 months, respectively, $p=0.003$).

B) Our score was then validated in the “Validation set”, including 116 patients registered prospectively and with available complete data. Patients with score 0 (20 patients, median OS not reached), had a significantly better survival when compared to patients with 1 or 2 poor risk features ($n=56$ and $n=40$, OS: 14 and 8.9 months, respectively, $p<0.0001$).

Table 1 . Clinical characteristics of 277 t-MN patients

Patient Characteristics	Retrospective series (n= 104)	Prospective series (n=173)	p
Median age – years	64 (27-83)	64 (21-87)	0.28
Sex (M/F)	44/60	73/100	1.0
Type of t-MN			0.3
AML (BM Blasts \geq 20%)	63	94	
MDS	41	79	
Primary disease:			
Lymphoproliferative diseases	35	71	
Breast	31	38	
Genito-Urinary	12	21	
Gastro-Intestinal	4	16	0.11
Thyroid	4	4	
Lung	4	5	
Other solid tumor	4	12	
Acute leukaemia	2	3	
Autoimmune disease	8	3	
Treatment of Primary diseases			
Chemotherapy	45	89	0.41
Radiotherapy	18	25	
Combined	41	59	
Median latency between primary therapy and t-MN diagnosis (years)	5.0 (0.5-32)	6.0 (0.7-48)	0.13
Karyotype (n=212)			
- Normal	28	48	
- Isolated chromosome 7 abnorm.	6	16	
- Complex	12	40	
- Balanced translocation*	6	4	0.05
- t(15;17)	9	7	
- Other abnormalities	16	20	
Median follow-up (months)	29.8 \pm 3	12.9 \pm 1	0.0001

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- t(9;11): 2 cases; t(11;16): 1; t (4;11): 1; t(9;16): 1; t (8;21): 3; t(3;8): 1; t(16;x)

Table 2 . Univariate Analysis for Overall Survival

Variable	Contrast	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Age at t-MN diagnosis	As continuous variable	0.0001	1.027	1.013	1.042
	≤ 60 vs > 60 years	0.0004	0.516	0.357	0.746
Previous	Hematologic vs solid tumor	0.5492	1.106	0.796	1.537
t-Mn Latency (years)	As continuous variable	0.7547	1.004	0.980	1.029
Previous radiotherapy	No vs yes	0.8518	0.970	0.701	1.341
Previous Chemotherapy	No vs yes	0.4401	0.823	0.502	1.349
Platelets	As continuous variable	0.0594	1.000	1.0	1.0
	≤ 30 vs > 31 10 ⁹ /L	0.0047	0.589	0.408	0.850
Haemoglobin	As continuous variable	0.0015	0.857	0.779	0.942
BM-Blasts	As continuous variable	0.5952	0.999	0.993	1.004
Karyotype	Favourable/intermediate vs unfavourable	<.0001	0.446	0.304	0.655
t-MN treatment	Standard CHT vs BSC	0.0063	1.873	1.194	2.939
	Hypomethylating vs BSC	0.3852	1.251	0.755	2.073
	Allogeneic SCT vs BSC	0.0110	0.445	0.238	0.831
	Autologous SCT vs BSC	0.5657	0.785	0.343	1.794

Significant values are indicated in bold.

CHT: standard induction chemotherapy; SCT: stem cell transplantation; BSC: best supportive care.

Table 3 . Multivariable analysis

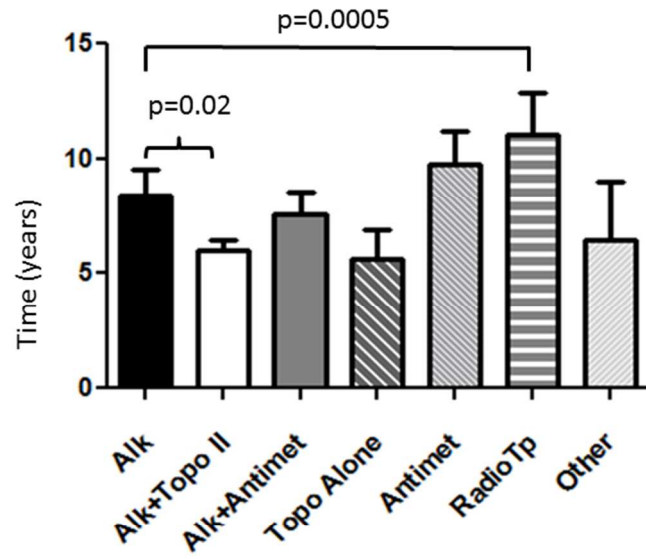
Parameter	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Age <60 vs >60 yrs	0.0075	0.526	0.328	0.842
Hb as continuous variable	0.0113	0.871	0.782	0.969
Karyotype (Favourable/intermediate vs Unfavourable)	0.0001	0.446	0.294	0.676

The multivariable analysis included variables significant in the univariate analysis and was stratified for t-MN treatment type, including best supportive care, standard chemotherapy, hypomethylating agents, autologous and allogeneic stem cell transplantation as stratification factors

Supplementary table 1. T-MN characteristics in patients with previous acute leukemia as previous primary disease

Primary acute leukemia (AL), FAB	Karyotype of primary AL	Karyotype of t-MN	Latency time (years)
APL	t(15; 17)	Complex	3,68
APL	t(15; 17)	Complex	2,49
ALL	normal	Complex	6,72
AML M2	t(8; 21)	Del(7q)	7,43
AML M4	Inv(16)	Del(7q)	3,32

APL: acute promyelocytic leukemia; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia

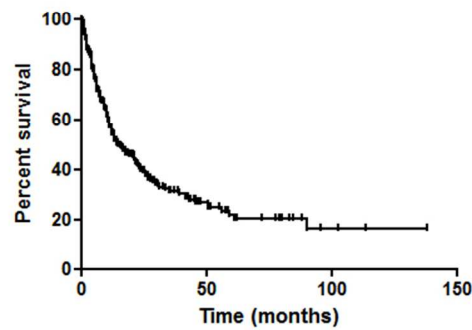


Fianchi et al, Figure 1

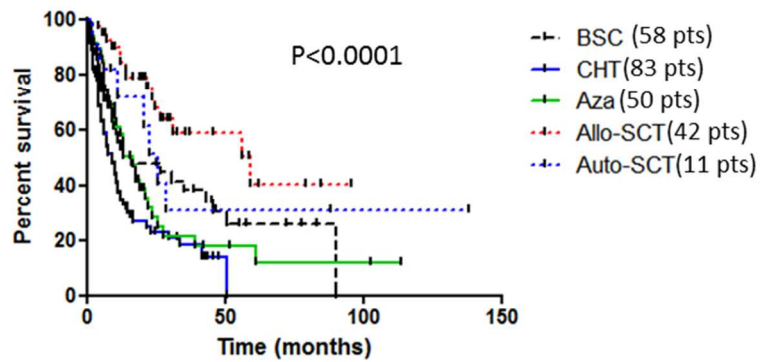
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AC

A) Overall Survival (277 t-MN)



B) Overall Survival according t-MN treatment

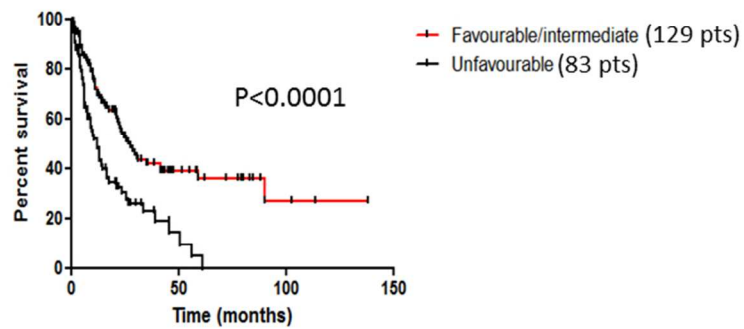


Fianchi et al Figure 2

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C) Overall Survival according karyotype



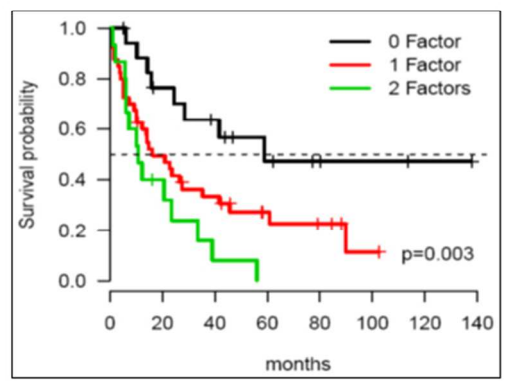
Pts= patients

Fianchi et al. Figure 2 c

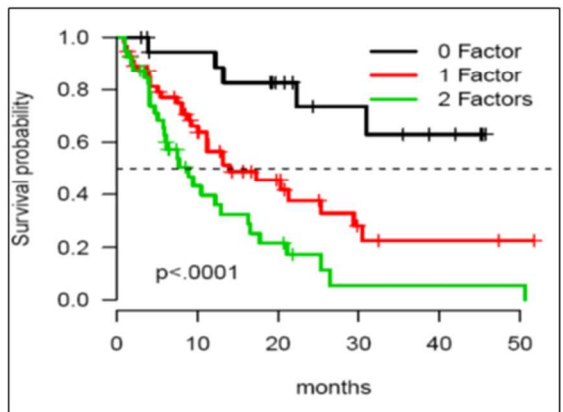
190x254mm (96 x 96 DPI)

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A) Overall survival according prognostic score in «training cohort»



B) Overall survival according prognostic score in «validation cohort»



Fianchi et al, Figure 3

Overall survival according prognostic score
190x254mm (96 x 96 DPI)

AC