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Response-Based Intensive Induction Chemotherapy of Curative Approach in Elderly Acute Myelogenous Leukemia Patients in Single Institution

Yoshiko Saito[1,2], Yoshiro Uzuka[1], Yhuka Takahashi[1] and Mari Ohtsuka[1]*

Department of Food and Nutrition, Miyagi Women's University, Japan

Abstract

To date, about 60-80% of adults with previously untreated de novo Acute Myeloid Leukemia (AML) enter Complete Remission (CR) when treated with standard regimen. However, such responses are rarely durable and relapsed when conventional therapy is administered. We aimed to establish a curable treatment protocol because prognosis after relapse is poor and fatal.

Over the last decades, progress in treatment for patients with AML, based on risk-directed stratification strategy, had brought large benefit to many patients. On the other hand, our treatment strategy was based on the fact that the assessment of treatment effect might help to define the prognosis of patient and possibly.

A total of 88 patients with de novo untreated AML treated between March 1995 and February 2011 was analyzed. Response-based intensive induction chemotherapy: single induction treatment consisted of priming standard regimen and additional induction regimen, which was continuedtill complete clearance of marrow leukemic blasts.

Results: By single induction strategy, CR was obtained in 21/21(100 %) of younger patients and in 19/20(95%) of elderly.8-week mortality was 0/21(0%) and1/20(5%) respectively. By an additional chemotherapy course, 8-week mortality was 7/24 (29.2%) in elderly. Differences between the single induction course and an additional courses at univariate analysis were statistically significant for CR ($p = 0.0053$) and for 8 week mortality ($p = 0.0031$).

Multivariate analysis of prognostic factors identified consistent independent poor prognostic factor for CR, 8-week mortality, and survival. These included age, unfavorable karyotypes, poor performance status, and abnormal organ functions. It was suggested that all risk factors may be overcome by the single induction strategy for patients with AML.

There was no co-relationship to the Charlson Comorbidity Index and the 8-week mortality. WT-1 measurements produced a dilemma, AML1/MTG8 and CBFβ/MYH11gene mutation measurement was the result which could be reflected in the chemotherapy.

Conclusions: Our unique "response-based single induction strategy" produces high CR rates over 95% and long-term Disease-Free Survival (DFS) over 70%. The intensified treatment was well tolerated in elderly as well as younger patients.

Keywords: Acute myeloid leukemia; Intensive induction chemotherapy; Blast cell clearance; End point of treatment; Elderly, MRD

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Correspondence:
Yoshiko Saito, Department of Food and Nutrition, Miyagi Women's University, Sendai Blood Disease Center, 28-3 Tomita aza Minamino-nishi, Taihakuku, Sendai City, Miyagi, Japan, Tel: 81-22-244-8558;
E-mail: sy33814@topaz.ocn.ne.jp

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Introduction

Many novel molecular targeting agents have been developed for acute myeloid leukemia but, still now, frontline therapy of acute myeloid leukemia has remained largely unchanged excluding the acute promyelocytic leukemia (APL) for several decades [1]. Standard regimen of Acute Myeloid Leukemia (AML) using anthracyclines and cytarabine for induction (3+7 regimen followed by various post-remission therapy has remained minor changed produce CR rates of 60% to 80%, With less than 20% of all patients achieving long-tsserm Disease-Free Survival (DFS) [2-4]. Over the last decade intensive induction chemotherapy produced the CR in the majority of adult patients with AML [5,6]. Despite these improvements, the long-term survival rate among patients who are less than 60 years of age is only 40%, and less than 10% of elderly patients with AML [2,7-10]. Unfortunately, the increase of dose intensity in elderly patients [3,5,11] is characterized by an increase

of CR rate and substantial increase of induction mortality rate [3,5-7]. There are several host and disease related predictive factors, among which adverse cytogenetics [6,12-15] and the presence of trans-membrane transport proteins [16]. Recent prospective randomized studies clearly demonstrate that elderly patients benefit from more intensive induction therapy [7,13,17-20] and particularly from full-dose application of anthracycline and possibly also cytarabine [6].

Kantarjean et al. [21] proposed prognostic models for CR, induction mortality and survival rates in elderly AML to establish baseline chemotherapy. They emphasized that cytogenetic studies have acquired major prognostic importance in the therapy of AML. The recent report of the Cancer and Leukemia Group B trials (9222) [22] tested the treatment intensification of AML in first remission with multiple sequential chemotherapy and high-dose cytarabine alone in younger patients, but there was no difference in DFS between the 2 regimens. Recent progress in treatment for patients with AML, based on risk-directed stratification strategy had brought large benefit to many patients [3,6,21]. Finally, recent report showed that intensity of the chemotherapy might be adapted to individual patient [23]. Some data showed no benefit of high-dose therapy and an autotransplant [24], while there is also the report that the high risk nature of the patients were favorable [1].

Our aim is to report remarkable results on intensive single induction strategy which provides insights to chemotherapeutic modality predictive for CR rates, induction mortality and survival. Intensified induction therapy could overcome almost all these adverse factors [25].

Minimal Residual Disease (MRD) analysis based on quantitative PCR of common fusion or mutated genes is gaining acceptance as a risk stratification tool and as a measure of impending relapse in AML. MRD is of clinical value in the assessment of response to chemotherapy, predicting relapse, and guiding therapeutic intervention [26-30]. Along with this report the importance of intensive remission induction therapy, for the determination of post remission and maintenance chemotherapy duration, the WT-1 and the fusion gene were measured with treatment courses [27,29,30].

Material and Methods

Patients

Eighty-eight consecutive patients age 16-88 years with de novo previously untreated AML were treated on intensive chemotherapy in Sendai Blood Disorder Center (SBDC) during March 1995 and February 2011. Patients with controlled co-morbid conditions were not excluded in this study. Patients with APL were excluded from this study. All patients provided written informed consent. Morphologic diagnosis of AML was made on May-Giemsa stained blood and bone marrow smears, and the diagnosis was confirmed by appropriate cytochemical staining, immunophenotyping by multicolored flow cytometry, and cytogenetics of leukemic cells. The disease was classified according to the French-American- British (FAB) classification system [31-33]. Performance status was assessed with the WHO criteria [34].

Karyotype was classified according to the International System for Cytogenetic Nomenclature. Favorable karyotypes were those with the abnormalities t(8;21),t(15;17) and inv (16). Unfavorable karyotypes were those with monosomy of chromosomes 5 or 7, deletion of the long arm of chromosome 5, abnormalities of the long arm of chromosome 3 or a complex karyotype (definedas more than three abnormalities). Patients with normal karyotype or with abnormalities other than those defined as favorable or unfavorable were classified as the intermediate cytogenetic group.

Treatment

At first, all consecutive patients received the priming induction therapy consisting of 40mg/m^2 daunorubicin by intravenous infusion on days 1-3 and 120 mg/m^2/day cytarabine by intravenous infusion daily by every 12 hour infusion on days1-7. On days 8 of induction therapy, the bone marrow was examined for the nucleated cell counts. When the result was the presence of blast cells, in response-based intensive induction strategy (SI), the addition of the medicine was carried out. That was continued till complete clearance of marrow leukemic blasts was obtained. Induction treatment was completed when the bone marrow nucleated cells <0.8 X 10^9 /L (corrected count) [35] in marrow aspirate with spicules, clearance of almost all blast cells (<2%) and the peripheral blood WBC count <0.6 X 10^9/L (end point for the completion of induction therapy-target point) was obtained. If the induction therapy could not reach to the target point, the induction course was subsequently continued till days 11 with daunorubicin 40mg/m^2/day and cytarabine 120mg/m^2/day as to reach to the target point by monitoring with every other day bone marrow examination. Generally, induction course was discontinued at 12 days to avoid later severe side-effects, and a second course of induction was usually not needed. After reaching to the target point, generally the bone marrow blast regeneration (>5% blast) did not reappear in weekly bone marrow sampling till CR. All younger patients who went into CR received 5 courses of consolidation therapy consisting of the same regimens at equivalent dose for 7 days. However in elderly patients 2-5 courses of consolidation therapy were given adjusting to myelotoxicity. Maintenance treatment consisting of 6 weekly courses of 30mg/m^2/d daunorubicin on days1 and 5 and 70mg/m^2/d cytarabine on days 1 to 5 was continued until molecular CR (2-5 courses) or a relapse. Close supportive care was given. All patients received intensive induction therapy in a laminar air flow room. For patients with decreasing absolute neutrophil counts(ANC) (<0.5 X 10^9/L), granulocyte colony stimulating factor (lenograstim: 5μg/kg/d or filgrastim: 6μg/kg/d) was administered till recovery to 1.0 X 10^9 /L ANC. Platelet transfusion was given for patients with decreased platelet count <10.0-20.0 X 10^9/L with hemorrhagic tendency. Supportive care included antibiotics and antifungal prophylaxis, blood product support.

Response criteria

A complete remission requires normalization of bone marrow with 5% or less blasts in aspirate samples with spicules and with a count of 500 nucleated cells, and peripheral neutrophil counts 1 X 10^9/L or above, and platelet counts100x10^9/L or above. There should be no blasts with Auer rods or persistence of extramedullary disease. Partial remission was defined as the persistence of marrow blast 5-20%. Patients with <5% blasts but with a hypo-cellular marrow precluding CR were also classified as being in PR. Failure was defined as marrow blasts >20. Cytogenetic complete remission was defined as reversion to normal karyotype at CR. Molecular CR was assessed using automated quantitative Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) technique [36] in case with a specific gene maker. Other molecular target, such as WT-1, was also assessed. Overall survival was defined as the time from the start of induction therapy to either death or last follow-up, censoring patients alive. Disease-Free Survival (DFS) was defined as the time from CR to either relapse or

Table 1: Patients characteristics.

Induction therapy	non-single		single		p
Number	43		45		
	19	24	24	21	
Age group	<60	60	<60	60	
(range years)	(16-59)	(61-88)	(18-59)	(63-82)	0.625
(median years)	(47)	(71)	(46)	(72)	
PS ECOG					0.013
1,2	12	10	20	10	
3,4	7	14	4	11	
FAB					
M0	1	3	0	1	
M1	12	15	8	11	
M2	3	2	9	5	
M4	2	0	3	3	
M5	1	2	2	0	
M6	0	2	2	1	
Karyotype					0.046
Faborable	4	2	9	4	
intermediate	12	12	14	11	
Unfaborable	3	9	1	3	
N.D.	0	1	0	3	
WBC ($X10^9$/L)					0.2193
<25	14	16	15	16	
25 - 100	5	4	7	2	
>100	0	4	2	3	

Abbreviations: PS: Performance Status; FAB: French-American-British Classification; ND: Not Determined

death in first CR, or last follow-up, censoring patients alive in first CR. 8-week mortality was related to treatment and/or hypoplasia. Cardiotoxicity was assessed using conventional cardio-echography and our original method: the phased tracking method [37,38].

Analysis for WT-1, AML1/MTG8 and CBFβ/MYH11 Gene Mutations

Available bone marrow samples and peripheral blood samples were obtained at diagnosis, post induction, post consolidation and during treatment-free remission. Total RNA extracted from bone marrow samples and peripheral blood samples by AGPC method. WT-1, AML1/MTG8 and CBFβ/MYH11 were determined by RT-PCR (EYELA) and real-time PCR (an ABI PRISM7700).

Statistical analysis

Differences among categorical covariates were evaluated using the chi-squared test. Response rates were compared in univariate analysis by the chi-squared test. Survival and remission duration curves were plotted by the Kaplan-Meier method and compared by the log rank test.

Multivariate analysis [33] of prognostic factors used the logistic regression methods for CR, induction mortality (8-week mortality) and Cox proportional hazard method for survival with standard methods using SAS ver. 8.02. Statistical significance is represented by two sided p values.

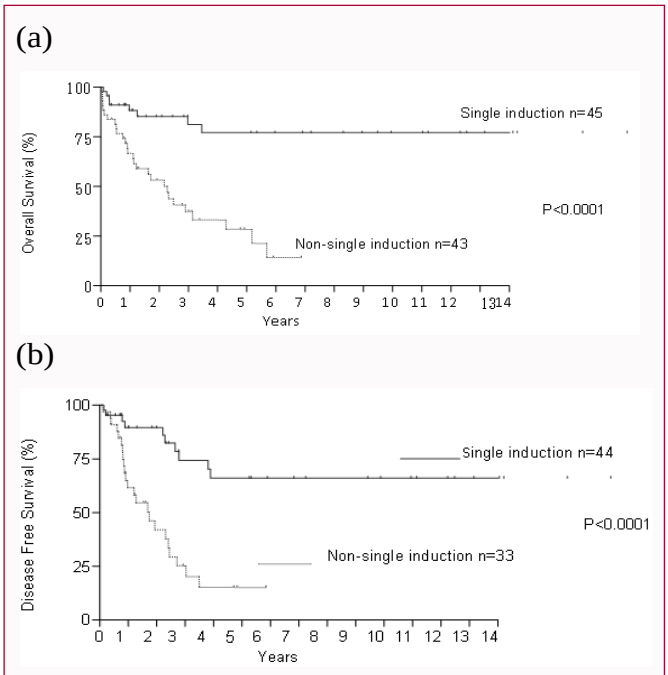

Figure 1: Kaplan-Meier analysis of induction therapy (a) overall survival (b) disease free survival.

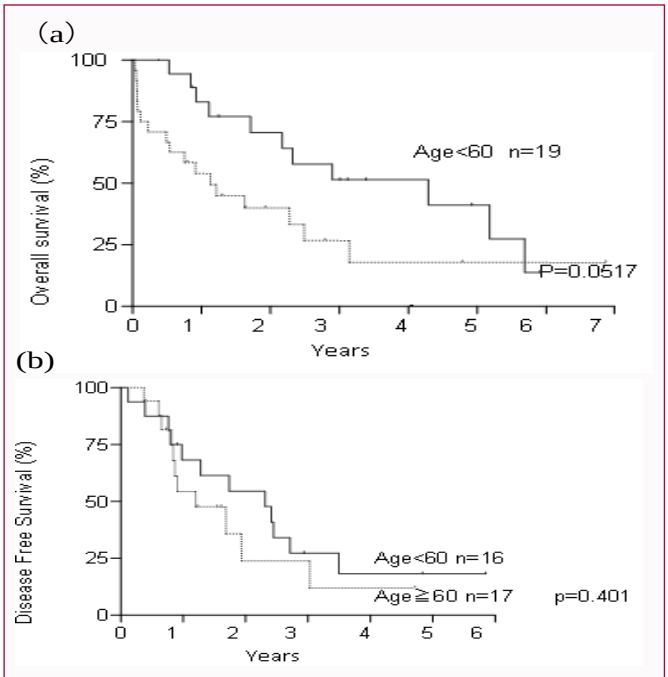

Figure 2: Non-single induction therapy compared with age category (a) overall survival (b) disease free survival.

Results

Presentation features of the entire patients were shown in (Table 1). Median follow-up of surviving patients is 5.1 years (range 0.3-14 years). Total of 88 patients were analyzed. Their median age was 66 years (range 16-88). Differences between the two groups for age, karyotypes, and WBC except PS were not statistically different.

Response to therapy

CR rate was16/19 (84.2%) in younger and 17/24 (70.8%) in elderly patients treated with non-single protocol (p = 0.0185) and 24/24

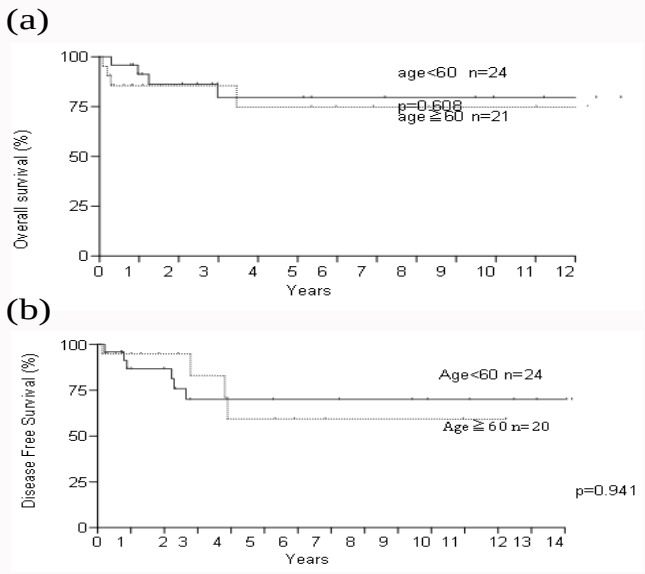

Figure 3: Single induction therapy compared with age category (A) overall survival (B) disease free survival.

(100%) and 20/21 (95.2%) respectively in single induction group (p = 0.2131). 8-week mortality was 0/19 and 7/24 (29.2%) respectively in non-single induction group(p = 0. 0053) and 0/24 and 1/21 (4.8%) respectively in single induction group (p = 0.213). Differences between the two groups at univariate analysis were statistically significant for CR (p = 0.0053) and for 8 week mortality (p = 0.0031). In non-single group, the overall survival was 41.0% in younger versus 13.3% in elderly (Figure 1a), and the 5-year DFS was 34.1% in younger versus 11.9% in elderly (Figure 1b). In the single induction group the 5-year survival rate was 72.4 % in younger 76.2% in elderly (Figure 2a) and the 5-year DFS was 72.8% and 69.6 % respectively (Figure 2b). Differences between the two groups at univariate analysis there were statistically significant differences for OS (p <0.0001) (Figure 3a) and DFS (p <0.0001) (Figure 3b). In non-single group, variables influencing response were age, PS and karyotype. There were significant differences in CR rates and substantial differences in long-term survival between both groups. In the single induction group, neither adverse cytogenetics nor PS were statistically significantly different for predictive of CR rate and long-term survival (Table 2). More than 60 years old 8-week mortality patients at eight, Wheatley [39] index were all patients poor. Two patients in 8-weekmortality with the Charlson comorbid index [40] were 3, other 6 were 0.

Toxicity

Toxicity showed in (Table 3). Hematologic toxicity was acceptable with around 15 days to recover to >0.5 X 10^9/L ANC in both younger adults and elderly patients among both groups(p = 0.2756). Median time to achieve an unsupported platelet count >30 X 10^9/L was around 15 days except in elderly patients treated with single induction therapy (p = 0.355). Severe Infectious complications (WHO grade 3,4) during induction therapy, in non-single induction group were observed in 14/43 (32.6%), whereas only 2/45 (4.4%) in single induction group (p = 0.0062). Cardiac functions were gradually decreased by the phased tracking method examination [37]. 6 out of 28 relapsed patients were fatal cardiotoxicity, but no relapsed patients were recovered cardiac function without additional chemotherapy [38].

MRD levels in chemotherapy courses

WT-1 and hybrid genes levels in the bone marrow and the peripheral blood samples collected over the course of chemotherapy were determined. WT-1 was expressed in 33 patients out of 39 at the time of diagnosis, 24 at the CR time and on 61.3% patients was expressed during disease free. Hybrid genes were expressed in all examined patients at the time of diagnosis. Patients sustained complete remission were negative, becomes positive before relapse (Table 4). TheWT-1 levels decreased as the treatment progressed were not predictive of the therapeutic efficacy, but re-elevation of the hybrid-gene level suggested relapse (Figure 4a and b).

Table 2: Treatment response according to induction and age group.

Induction therapy	single	non-single	p
Age group			
8 week mortality %	2.2	14	0.0031
CR rate %	97.8	76.7	0.0053
DFS weeks*	4-730+(/)	6-305+(88)	<0.0001
OS weeks*	4-730(/)	2-358(113)	<0.0001
Induction therapy	Single		p
Age group	<60	60	
Number	24	21	
8 week mortality %	0	4.8	0.2131
CR rate %	100	95.2	0.2131
DFS weeks*	12-730+(/)	5-638+(/)	0.942
OS weeks*	16-730+(/)	5-642+(/)	0.608
Induction therapy	Non-single		p
Age group	<60	60	
Number	19	24	
8 week mortality %	0	25	0.0053
CR rate %	84.2	70.8	0.0185
DFS weeks*	6-305+(91)	20-246+(48)	0.401
OS weeks*	20+-309+(177)	2+-358+(48)	0.0517

Table 3: Toxicities WHO Grade ≧3.

Induction therapy	non-single	single	p
	n(%)	n(%)	
Neutropenia	43/43(100)	45/45(100)	/
Thrombocytopenia	43/43(100)	45/45(100)	/
Infection	14/43(32.6)	2/45(4.4)	0.0017
Hepatotoxicity	0/43	0/45	/
Nephrotoxicity	2/43(4.7)	0/45	0.47
Neurotoxicity	0/43	0/45	/
Late cardiotoxicty	0/43	0/45	/

Table 4: Monitaring results of WT-1, AML1/MTG8 and CBFβ/MYH11.

	WT-1			AML1/MTG8 and CBFβ/MYH11		
	No of Cases			No of Cases		
	$>10^{-3}$	10^{-3} - 10^{-5}	$<10^{-5}$	$>10^{-3}$	10^{-3} - 10^{-5}	$<10^{-5}$
at Diagnosis	33	0	6	58		
at CR	21	3	9	2	3	14
at relapse	14	0	6	20	0	19
stll CR	19	0	12	0	0	19

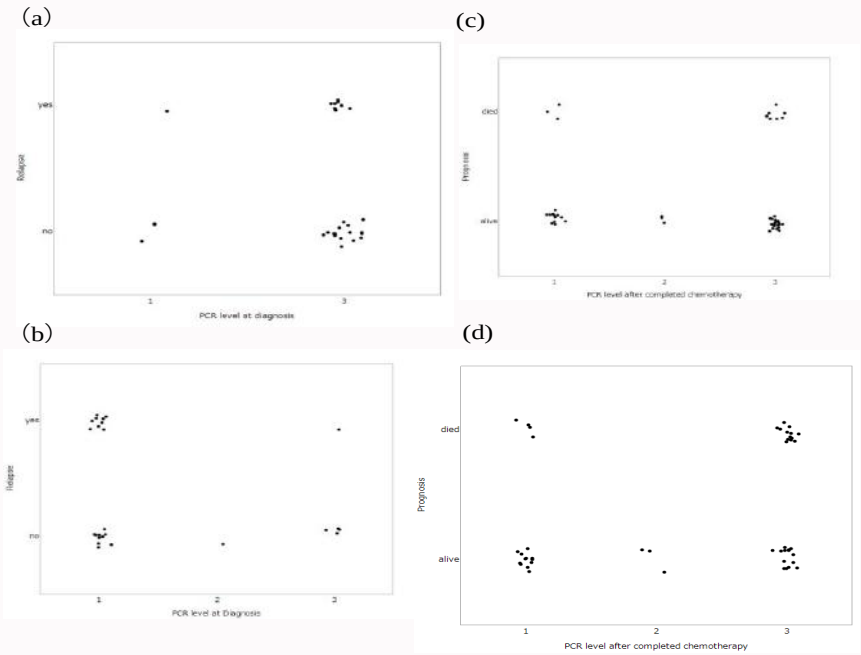

Figure 4: Relationship between PCR level and outcomes.
(A) Relationship between WT-1 PCR level at diagnosis andrelapse
(B) PCR level at diagnosis of Hybrid genes *vs.* relapse
(C) WT-1 level after completed chemotherapy vs prognostic outcomes
(D) PCR levels of after completed chemotherapy vs prognostic outcomes
PCR levels 1: $<10^{-5}$, 2: 10^{-5}~10^{-3}, 3: $>10^{-3}$

Discussion

In1976, we proposed the DCMP 2-step Therapy[41]for induction therapy of AML. In our early DCMP 2-step therapy, induction treatment was divided into 2 courses with short interval (within 7-14 days). The CR rate of more than 80% obtained by this strategy was one of excellent results at that decade [34]. Japanese Leukemia Study Group (JALSG) has started on the conception of this strategy with some modifications. However, long-term result was disappointing (5-year DFS only 15%) by this strategy. The cause of failure to achieve long-term outcome was considered as following: in patients with AML, leukemic blast cell regeneration occurred rapidly with changing cell-kinetics of leukemic blasts during and after chemotherapy [42]. A malignant cell population that has survived initial induction treatment might show resistance to chemotherapy due to special genetic or kinetic changes. In contrast, early induction therapy deals with naïve tumor cells possibly different from the counterparts after chemotherapy in terms of their kinetic status and sensitivity to chemotherapy [43]. In particular, older patients have many problems due to leukemic cell drug resistance and decreased tolerance of the side-effects of therapy. In 1991 we stared intensive chemotherapy for elderly patients as well as younger adults with AML using the response-oriented intensive induction chemotherapy [44]. Remarkable improvement has been obtained in terms of CR, however, improvement of long-term survival was not satisfactory. Thus, we have started non-single induction therapy based on new target point of induction therapy in 1995. However, even by this method, high incidence of early chemotherapy death was not avoided, particularly, in older patients. By this strategy intensive induction was based on prolongation of duration and increasing of dose of cytarabine alone. Based on many previous experiences and according to some literaturs [23,45-47], we get the courage with the confidence that early blast cell clearance by early single induction therapy is most important for both achievement of CR and long-term outcome in both younger and elderly with AML. Thus we have developed the unique responce0oriented intensive "single induction strategy" with a curative intent for elderly as well as younger patients with AML in 2000. The aim of the single induction strategy is to minimize leukemic blasts by early blast clearance after one course of induction therapy, suppression of early re-proliferation of residual leukemic blasts during and after bone marrow aplasia, the decrease in severe myelotoxicity induced by prolonged induction chemotherapy. To suppress the re-proliferation of residual leukemic blast during early induction therapy is essential for killing the naive tumor cells, resulting in long-term remission or cure in patients with AML. It was reported that elderly patients showed a great benefit from full-dose application of standard induction regimen than from less intensive chemotherapy [6,17,47]. In addition, toxicity may be reduced if patients require no more than one cycle of induction therapy to achieve complete remission [46,48]. Generally, hematopoietic precursors are considered to be designed to survive the repeated exposure to environmental toxins encountered during lifetime. As demonstrated by marrow purging experiments, the hematopoietic stem cell can survive an exposure to very large doses of cytotoxic agents *in vitro* [21]. Thus hematopoietic precursors in the bone marrow of elderly patients might survive early exposure to intensive induction therapy. However, in the elderly patients, reserve function of bone marrow might be reduced probably by the detrimental effects of stem cell aging [45,49,50] and exhaustion of primitive stem cell after chemotherapy. So, bone marrow stem cell function is more vulnerable to delayed and repeated exposure to chemotherapeutic regimens. In addition the leukemic cell in older patients arises from a more proximal pluripotent stem cell in the hematopoietic hierarchy than is the case for younger adults with the disease [3,5-7]. By its

very nature, this proximal stem cell is intrinsically more resistant to chemotherapy. We considered that chemo-therapeutic effects might help to define the prognosis of the individual patient and possibly might adapt the intensity of the chemotherapy to the individual therapeutic response. The single induction strategy in very early phase of treatment may not only minimize residual disease, but also reduce substantial myelotoxicity. For this purpose, we decided the end point of the first induction chemotherapy as to reach target point by monitoring with repeated bone marrow examination at day 8-11 of induction treatment. Inclusively, our therapeutic modality is not only adapted to individual response but also adapted to individual toxicity for dose decision making which could overcome all adverse factors. Elderly as well as younger adults have excellent outcomes. In addition, single induction therapy introduces a more standardized approach that achieves homogeneity in the quantity of induction treatment for tumor cell burden. There was no patient's refusal of intensive chemotherapy. Our study shows that the aggressive approach was feasible in all elderly as well as younger adults.For patients wishing chemotherapy without feeling frailty until just before leukemia diagnosis with a high possibility of early mortality index and CCI [40], further research and investigation is necessary to select chemotherapy.

The prognostic impact of the normalized bone marrow and peripheral blood WT-1 levels at diagnosis, post-induction and post intensification was limited on the our data but hybrid gene mutations levels reflected the leukemic mass. But further investigation is needed in order to reflect chemotherapy courses reduction or extension [26,27,51]. MRD may serve as individualized chemotherapy options of the consolidation and the maintenance therapy.

Conclusion

The most important thing to get a good long-term survival is to achieve a complete remission with one course of remission induction therapy. Our response-based intensive induction strategy is feasible and appropriate.

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