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Potent Graft-versus-Leukemia Effect after Reduced-Intensity Allogeneic SCT for Intermediate-Risk AML with *FLT3*-ITD or Wild-Type *NPM1* and *CEBPA* without *FLT3*-ITD

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To investigate the role of reduced-intensity allogeneic (RIC-allo) stem cell transplant (SCT) as postremission therapy in adult intermediate-risk patients with acute myelogenous leukemia (AML) with *FLT3*-ITD or wild-type *NPM1* and *CEBPA* without *FLT3*-ITD, we conducted a single-center retrospective study between January 2001 and December 2010. Sixty-six patients were included: 37 treated with RIC-alloSCT and 29 with nonallogeneic SCT therapies. Both groups were comparable concerning age, WBC count at diagnosis, gender, karyotype, genotype, and number of courses of chemotherapy to reach complete remission (CR1). Median follow-up after CR1 was 37 months (range, 11-112 months) and 48 months (range, 9-83 months) in the allo and no-allo groups, respectively. In the allo versus no-allo groups, the 3-year cumulative incidence of relapse (CIR) rates were 25% ± 8% versus 61% ± 9%; $P = .005$. The 3-year nonrelapse mortality (NRM), overall survival (OS), and relapse-free survival (RFS) were 22% ± 7% versus 4% ± 4% ($P = .005$), 52% ± 9% versus 44% ± 10% ($P = .75$), and 53% ± 9% versus 35% ± 9% ($P = .28$), respectively. Multivariate analysis indicated that CIR was reduced by allo (hazard ratio [HR], 0.32; $P = .01$). A landmark analysis performed at day 185 after CR1 confirmed a lower CIR after allo. RIC-allo reduces the risk of relapse, suggesting a potent graft-versus-leukemia (GVL) effect in these patients at a high risk of relapse.

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INTRODUCTION

The role of allogeneic stem cell transplant (alloSCT) in adults with intermediate-risk acute myeloid leukemia (IR-AML) in first complete remission (CR1) is controversial and remains a domain of intense investigation [1-4]. A recent meta-analysis of prospective clinical trials has reported a significant benefit of myeloablative allo for relapse-free survival (RFS) and overall survival (OS) [5]. The median age of patients in most of these trials was in the 30s, and an equivalent benefit in older patients remains uncertain. A German-Austrian retrospective study has demonstrated that genotypes defined by the mutational status of FMS-like tyrosine kinase 3 (*FLT3*), nucleophosmin1 (*NPM1*), and CCAAT/enhancer binding protein α (*CEBPA*) genes were associated with the outcome for cytogenetically normal AML [6]. The benefit of allo was limited to the subgroup of patients with the prognostically adverse genotype *FLT3* internal tandem duplication (*FLT3*-ITD) or the genotype consisting of wild-type *NPM1* and *CEBPA* without *FLT3*-ITD (triple-negative). In these patients, allo improved RFS. It must be emphasized that patients were under

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60 years of age, and underwent transplantation with an HLA matched-related donor (MRD) after a myeloablative conditioning regimen. As a consequence, the benefit of reduced-intensity allo (RIC-allo) as postremission therapy in older patients with IR-AML and *FLT3*-ITD or a triple-negative genotype remains uncertain. In an effort to further explore the role of allo in this setting, we performed a retrospective study of patients treated with RIC-allo or nonallogeneic SCT therapies in the absence of a suitable donor. Our aim was to compare both postremission strategies.

MATERIALS AND METHODS

Selection of Patients

The selection criteria for inclusion in this study were set to select a population of patients between 18 and 65 years of age, diagnosed with de novo AML (except acute promyelocytic leukemia) between January 2001 and December 2010 at our center. All patient records were reviewed, and some patients were excluded from the analysis as detailed in Figure 1. AML with favorable or adverse karyotypes were excluded. Patients transplanted in CR1 after a myeloablative conditioning regimen were also excluded, as were patients who never reached complete remission (CR). Genetic-risk groups were defined according to the recommendations from an international expert panel [7]. Patients diagnosed before 2007 at our center were not genotypically defined at diagnosis, and those with available frozen leukemic cells were retrospectively analyzed. Thus, patients with a normal karyotype and either *FLT3*-ITD or triple-negative genotype (intermediate-I group) were included in the present study, as were patients with cytogenetic abnormalities not classified as favorable or adverse and either *FLT3*-ITD or triple-negative genotype (intermediate-II group). We have included patients with cytogenetic abnormalities not classified as favorable or adverse when associated with an adverse genotype because there is some evidence that *FLT3*-ITD and triple-negative genotype adversely affect the outcome of these patients [7-9] as they do for patients with a normal karyotype [6]. Before 2007, our therapeutic strategy was to pursue allo in CR1 for patients with cytogenetically defined IR-AML. From 2007, the same strategy was applied for patients with IR-AML with either *FLT3*-ITD or triple-negative genotype. Patients with cytogenetic abnormalities not classified as favorable or adverse and a favorable genotype (mutated *NPM1* without *FLT3*-ITD or mutated *CEBPA*) were not included in our comparative study because we always chose to treat them with nonallogeneic SCT therapies in CR1 without looking for a donor. As a consequence, the unique reason for not performing allo in our study was the absence of a suitable donor at the time of CR1. From 2001 to 2006, patients underwent transplantation only with MRDs. From 2007, patients underwent transplantation in priority with MRD, then matched-unrelated donor, and finally mismatched-unrelated donor (C or DQB1) in the absence of MRD or matched-unrelated donor. Cord blood units were used from 2008 in the absence of any related or unrelated donor. Finally, to minimize potential biases favoring patients who underwent transplantation, patients ineligible for allo because of a poor performance status or a severe comorbidity were excluded, as were patients deceased or in relapse before the median time between CR1 and allo.

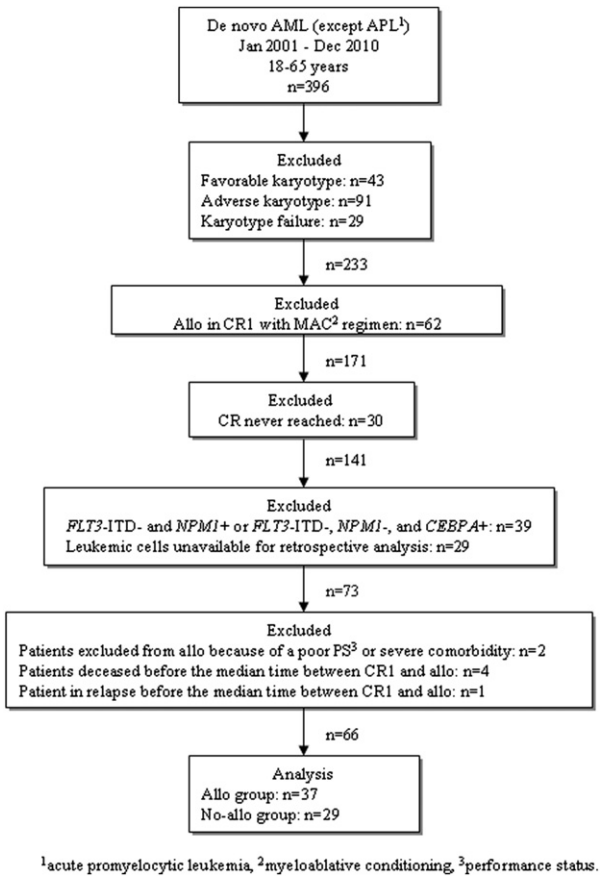

Figure 1. Selection of patients included in the study.

Materials

Bone marrow samples were used whenever available. In all other cases, peripheral blood samples were examined if the percentage of blasts in peripheral blood was >25%. Genomic DNA was extracted from mononuclear cells separated by Ficoll gradient. Genomic DNA (gDNA) was extracted using a QIAamp DNA Blood miniKit (Qiagen, Courtaboeuf, France), according to the manufacturer's protocol.

Detection of *FLT3*-ITD, *NPM1*, and *CEBPA* Gene Mutations

The presence of *FLT3*-ITD and *NPM1* gene mutations was detected by capillary electrophoresis-based fragment analysis after fluorescence labeled PCR on gDNA. Primers 11F and 12R were used for *FLT3*-ITD detection as previously described [10]. Forward primer 5′-[HEX]TTCCATACATACTT AAAACCAAGCA-3′described by Boissel et al. [11] and reverse primer 5′-TTAACTCTCTGGTGGTA GAATGAA-3′ described by Falini et al. [12] were used for *NPM1* exon 12 mutations. Mutations of the *CEBPA* gene were detected by gDNA PCR and direct sequencing according to the method previously described [13].

Patients' Characteristics

Sixty-six patients were included. After CR1, 37 were treated with RIC-allo (allo group) and 29 with non-allogeneic SCT therapies (no-allo group). The reasons for choosing RIC instead of myeloablative conditioning regimen were as follows: age ≥50 years (n = 32), number of courses of chemotherapy before allo ≥3 (n = 4), and abnormal liver function tests (n = 1). The median age at diagnosis was 55 years (range, 19-64 years). There were 33 male patients. Forty-three patients had a normal karyotype and 23 had cytogenetic abnormalities not classified as favorable or adverse. Forty-two patients had a triple-negative genotype and 24 patients had the *FLT3*-ITD. The median time between CR1 and allo was 114 days (range, 24-295 days). Conditioning regimen combined fludarabine with an alkylating agent (n = 27) or TBI2Gy (n = 10). The sources of stem cells were peripheral blood (n = 32), bone marrow (n = 1), or cord blood (n = 4). Donors were matched-related (n = 18), matched-unrelated at the allele level (4 digits) for HLA-A, B, C, DRB1, and DQB1 (n = 11), or mismatch-unrelated (C: n = 2 and DQB1: n = 2, all at the antigen level).

Statistical Analysis

Patient-related and disease-related variables of the 2 groups (receiving or not allo) were compared using the chi-square statistic for categorical and the Mann-Whitney test for continuous variables. Variables considered were allo (yes versus no), patient age at diagnosis (≥55 versus <55 years, because this is the median age of the whole cohort), WBC count at diagnosis (> versus <30,000/μL) [14], karyotype (normal versus abnormal), genotype (*FLT3*-ITD versus triple-negative), number of courses of chemotherapy to reach CR1 (1 versus ≥2), and year of CR1 (> versus ≤2006). RFS after CR1 was defined as survival without evidence of relapse or progression. The nonrelapse mortality (NRM) was defined as death while in CR. Cumulative incidence curves were used for relapse incidence (RI) and NRM in a competing risks setting, death in CR being a competing event for relapse. The Gray test was used for univariate comparisons [15]. Probabilities of OS and RFS were calculated using the Kaplan-Meier estimate [16]; the log-rank test was used for univariate comparisons. In order to take into account for delay before allo, we performed a landmark analysis at day 185 after CR1. All factors studied were included in the Cox proportional hazards [17] for OS and RFS, and in a Fine-Gray model for RI and NRM [18]. Given the retrospective design of the study, and in order to minimize all possible biases, we decided to adjust the comparison between the 2 groups (allo versus no-allo) on all potential prognostic factors even if not significant in our population. All tests were 2-sided. The type I error rate was fixed at 0.05 for determination of factors associated with time-to-event outcomes. Statistical analyses were performed with the SPSS version 19 (IBM, Chicago, IL) and R 2.13.2 software packages (R Development Core Team, Vienna, Austria).

RESULTS

Comparison of Patients' Characteristics between Allo and No-Allo Groups

As shown in Table 1, both groups were comparable concerning age, gender, WBC at diagnosis, proportion of normal/abnormal karyotypes, proportion of *FLT3*-ITD/triple-negative genotypes, and number of courses of chemotherapy to reach CR1.

Relapse and Survival

The median follow-up after CR1 was 37 months (range, 11-112 months) in the allo group and 48 months (range, 9-83 months) in the no-allo group. In the allo group, the median follow-up after transplantation was 30 months (range, 7-108 months). In the allo versus no-allo groups, the 3-year OS were 52% ± 9% versus 44% ± 10%, $P = .75$, and the 3-year RFS were 53% ± 9% versus 35% ± 9%, $P = .28$ (Figure 2). Nineteen patients have died in the allo group from disease (n = 9), infections (n = 7; bacterial septic shock: n = 3; bacterial pneumonia: n = 3; cytomegalovirus colitis: n = 1, with 5 patients having extensive chronic graft-versus-host disease [GVHD] and 1 acute [aGVHD] at the time of infection), aGVHD (n = 2), or suicide (n = 1). Fourteen patients have died in the no-allo group from disease (n = 13) or infection (n = 1). Neither OS nor RFS were significantly influenced by any of the studied variables in univariate or multivariate analyses. In the allo versus no-allo groups, the 3-year cumulative incidence of relapse (CIR) rates were 25% ± 8% versus 61% ± 9%, $P = .005$ (Figure 3). In the allo group, 10 patients have relapsed

Table 1. Characteristics of Patients Included in the Study Separated into Allo and No-Allo Groups

Variables	Allo Group n = 37	No-Allo Group n = 29	*P* Value
Age at diagnosis, years			
Median (range)	56 (31-64)	54 (19-64)	.50
Gender			.08
Male	22	11	
Female	15	18	
WBC count >30,000/μL at diagnosis	11/37	11/29	.50
Karyotype			.30
Normal	22	21	
Abnormal	15	8	
Genotype			.20
FLT3-ITD	11	13	
Triple-negative	26	16	
Courses of chemotherapy to reach CR1 (n)			1 versus ≥2
1	22	21	.30
2	12	8	
3	3	0	
Courses of chemotherapy after CR1 (n)*			NA
0	8	0	
1	15	3	
2	11	6	
≥3	0	8	
1 + autoSCT	3	8	
2 + autoSCT	0	4	

Allo indicates allogeneic; autoSCT, autologous stem cell transplantation; NA, not applicable.
*Before allogeneic SCT in the allo group.

at a median time of 8 months (range, 4-39 months) after CR1. In the no-allo group, 18 patients have relapsed at a median time of 8 months (range, 4-44 months) after CR1. Six of them were treated with allo in CR2. The other patients did not undergo transplantation mainly because of inability to reach CR2 (n = 7), absence of a donor (n = 4), or ineligibility by age >65 years at relapse (n = 1). In the allo versus no-allo groups, the 3-year NRMs were 22% ± 7% versus 4% ± 4%, $P = .005$. Univariate analyses indicated that CIR was reduced only by allo in CR1. Multivariate analysis for CIR indicated that allo was associated with a reduced risk of relapse (hazard ratio [HR], 0.32; 95% confidence interval [CI]: 0.14-0.76; $P = .01$; Table 2). Univariate and multivariate analyses indicated that NRM was increased only by allo in CR1. Multivariate analysis for NRM is shown in Table 2.

One patient only has undergone transplantation after day 185 after achievement of CR1, and 6 patients relapsed or died before this date (3 in the allo group and 3 in the no-allo group). By a landmark analysis 185 days after achievement of CR1, the 3-year CIRs were 19% ± 7% in the allo group versus 57% ± 11% in the no-allo group ($P = .003$). NRM was 23% ± 8% in the allo group versus 4% ± 4% in the no-allo group ($P = .005$). The 3-year OS was 57% ± 9% versus 49% ± 11% ($P = .70$) and 3-year RFS was 58% ± 9% versus 39% ± 10% ($P = .30$) in the allo and no-allo groups, respectively. By multivariate analysis with landmark at day 185, CIR was reduced in the allo group compared with the no-allo group (HR, 0.23; 95% CI: 0.09-0.59; $P = .003$), and NRM was higher in the allo group compared with the no-allo group (HR, 9.9; 95% CI: 1.22-80; $P = .03$).

Graft-versus-Host Disease

The 2-year cumulative incidence rates of aGVHD grade II to IV, aGVHD grade III to IV, and extensive chronic GVHD were 40% ± 8%, 16% ± 6%, and 25% ± 7%, respectively.

DISCUSSION

In the present study, we report a reduced CIR after RIC-allo as postremission therapy in patients with de novo IR-AML and an unfavorable genotype. The high incidence of relapse observed in our study in the no-allo group is comparable to incidence rates reported in previous studies [6,8,9]. Moreover, the NRM observed in our study in the allo group is also comparable to NRM reported in studies focusing on RIC-allo in myeloid malignancies [19]. During the most recent years of the study, data on allo with

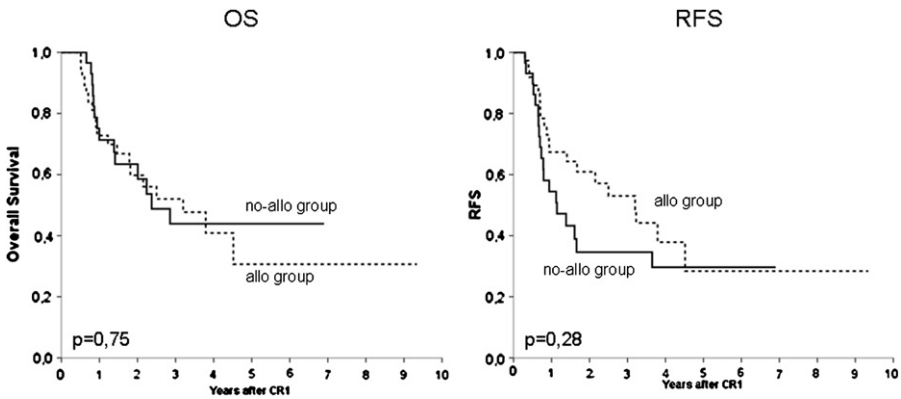

Figure 2. Overall survival (OS) and relapse-free survival (RFS) in allogeneic (allo) and no-allo groups.

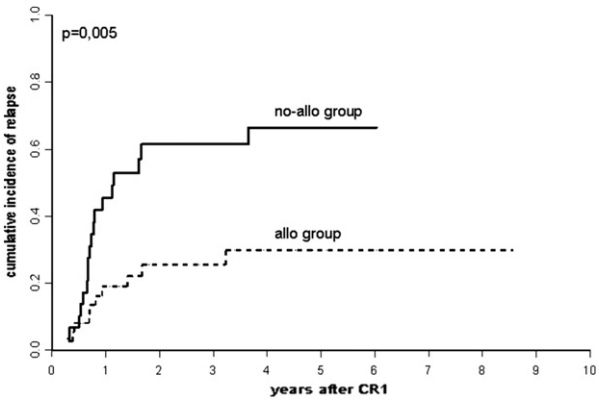

Figure 3. Cumulative incidence of relapse (CIR) in allogeneic (allo) and no-allo groups.

unrelated donors have emerged, as well as clinical trials with cord blood, accounting for a higher number of allo in recent years, and consequently a slightly shorter follow-up in the allo group. Because only RIC regimes were used in our study, the reduced incidence of relapse suggests a potent graft-versus-leukemia (GVL) effect. This benefit did not translate into a difference in survival because allo was associated with a higher NRM and because some successful allo procedures were performed in CR2 in the no-allo group.

The cytogenetic analysis at diagnosis provides the most powerful independent prognostic factor in AML [14]. The prognosis has been refined in recent years by analysis of gene mutations in *FLT3*, *NPM1*, and *CEBPA* [8,9,20,21]. These efforts have led to the characterization of IR-AML with a normal karyotype or cytogenetic aberrations not classified as favorable or adverse, associated with *FLT3*-ITD, or triple-negative genotype [6-9]. The majority of these patients reach a CR, but a high number rapidly relapse after usual consolidation therapy with high-dose cytarabine, accounting for a significant impact on relapse risk and OS. This adverse effect is more striking in the presence of a high *FLT3*-ITD allele ratio indicative of a homozygous mutation [9]. However, this last data has not been incorporated into the standardized reporting system for genetic abnormalities recently recommended from an international expert panel [7]. Naturally, these observations raise the question of the optimal postremission therapy, but prospective studies are lacking. Koreth et al. [5] reported the results of a systematic review and meta-analysis of prospective trials evaluating allo versus non-allogeneic SCT therapies for AML in CR1, based on donor availability. The risk groups were cytogenetically defined. They reported that allo had significant RFS and OS benefit for IR-AML. Most patients were young, with median ages in the 30s, and underwent transplantations with an MRD after a myeloablative conditioning regimen. To further refine the analysis, Schlenk et al. [6] evaluated the associations of the mutations of the *NPM1*, *FLT3*, *CEBPA*, *MLL*, and *NRAS* genes with clinical outcomes in 872 adults younger than 60 years of age with cytogenetically normal AML. In that study, RFS was significantly longer in patients who underwent transplantation in the subgroup of patients with the prognostically adverse genotypes *FLT3*-ITD or triple-negative. Patients underwent transplantation with an MRD after a myeloablative conditioning regimen. Additionally, Dezern et al. [22] reported the outcome of allo in adult patients under 60 years of age with AML and *FLT3*-ITD. The study included 133 patients. Among them, 31 harbored an *FLT3*-ITD mutation at diagnosis. The OS for the patients with *FLT3*-ITD was comparable to the 102 patients with wild-type *FLT3* over the same 4-year time period. Historically, OS for patients with *FLT3*-ITD AML was significantly worse than for patients with AML lacking this mutation. The authors hypothesized that the difference that might have contributed to the surprisingly favorable outcomes for the *FLT3*-ITD group was their aggressive pursuit of allo. Altogether, and despite the lack of prospective data, these studies suggest that younger patients with *FLT3*-ITD or triple-negative IR-AML benefit from myeloablative allo as postremission therapy. Unfortunately, the available data do not indicate if this benefit is mostly due to the antileukemic activity of the conditioning regimen, or the existence of a potent GVL effect, or both.

A beneficial effect of RIC-allo in older patients with IR-AML remains uncertain. As summarized in a recent review article by Storb [19], several authors have demonstrated the feasibility of RIC-allo in older

Table 2. Results of Multivariate Analyses for CIR and NRM

	CIR		NRM	
Variables	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Allo (yes versus no)	0.32 (0.14-0.76)	.01	9.71 (1.18-80)	.04
WBC at diagnosis (> versus <30,000/μL)	1.27 (0.45-3.57)	.65	0.8 (0.17-3.73)	.77
Karyotype (normal versus abnormal)	1.25 (0.6-2.59)	.56	0.73 (0.23-2.25)	.58
Genotype (FLT3-ITD versus triple-negative)	1.1 (0.4-3.17)	.85	0.74 (0.13-4.22)	.73
Age at diagnosis (≥ versus <55 years)	1.85 (0.83-4.12)	.13	1.41 (0.32-6.13)	.65
Courses of chemotherapy to reach CR1 (1 versus ≥2)	0.64 (0.28-1.49)	.30	1.47 (0.42-5.17)	.54
Year of CR1 (> versus ≤2006)	0.87 (0.35-2.2)	.77	0.75 (0.23-2.39)	.62

CIR indicates cumulative incidence of relapse; NRM, nonrelapse mortality; HR, hazard ratio; CI, confidence interval; Allo, allogeneic.

patients using related or unrelated donors, with reasonable outcomes. That review indicated that 2 to 5-year survival rates of 25% to 64% could be expected, with similar survival for recipients of related and unrelated HLA-matched grafts. Relapse rates ranged from 16% to 53%, and the major issue was NRM ranging from 16% to 39%. No RIC regimen has proven its superiority, and fludarabine combined with either low-dose TBI or an alkylating agent usually leads to comparable outcome [19]. It must be emphasized that no study has explored the outcome of RIC-allo in the specific population of patients with *FLT3*-ITD or triple-negative IR-AML. As a consequence, the benefit of RIC-allo in these patients remains uncertain to a large extent.

It must be acknowledged that the retrospective nature of our study precludes the declaration of any firm conclusions. The absence of difference in OS and RFS must indeed be interpreted with caution given the modest size of the study. However, we report that RIC-allo reduces the risk of relapse in patients with *FLT3*-ITD or triple-negative IR-AML in CR1, suggesting a potent GVL effect. This finding can be discussed in light of a recent article suggesting that the *FLT3*-ITD-mutated receptor is hyper-responsive to its cognate ligand rather than autonomously activated [23]. As chemotherapy leads to high levels of cognate ligand during the period of recovery and during consolidation, the author raises the provocative hypothesis that successive courses of consolidation chemotherapy could promote relapse. If this hypothesis is confirmed, and considering the GVL effect suggested in our study, the best postremission strategy in patients with *FLT3*-ITD could be to proceed as rapidly as possible to allo once remission is achieved. A strategy of early allo might also permit the decrease of NRM and thus improve the outcome of these patients.

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