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Steroid treatment of acute graft versus host disease grade I: a randomized trial

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Running title: Steroid treatment of GvHD grade I

ABSTRACT

Patients with acute graft versus host disease (GvHD) grade I, were randomized, to an *observation arm (n=85)* or to a *treatment arm (n=86),* consisting of 6-methylprednisolone 1 mg/kg/day , after stratification for age and donor type. The primary end point was development of grade II-IV GvHD. The cumulative incidence of GvHD grade II-IV, was 50% in the *observation* and 33% in the *treatment* arm (p=0.005). However, grade III-IV GvHD was comparable (13% vs 10% respectively; p=0.6), and this was true for sibling and alternative donor transplants. Moderate/severe chronic GvHD was also comparable (17% vs 19%). In multivariate analysis, an early interval between transplant and randomization (≤ day +20) , was the only negative predictor of GvHD grade III-IV .Patients in the *observation* arm had less infectious bacterial episodes (12 vs 25, p=0.04), less severe infectious fungal episodes (0 vs 3; p=0.04), and less severe adverse events (3 vs 11 p=0.07). At 5 years, non relapse mortality was 20% (*observation*) vs 26% (*treatment*) (p=0.2), relapse related death 25% vs 21%, and actuarial survival was 51% vs 41% respectively (p=0.3). In multivariate analysis, advanced disease phase, older age and an early onset of GvHD, were significant negative predictors of survival, independent of the randomization arm.

In conclusion, steroids treatment of acute GvHD grade I prevents progression to grade II but not to grade III-IV GvHD, and there is no effect on non relapse mortality and survival; patients treated with steroids, are at a higher risk of develping infections and have more adverse events. (This trial is registered as EUDTRACT 2008-000413-29)

INTRODUCTION

There is uncertainty as to whether acute graft versus host disease (GvHD) grade I, that is a skin rash over <50% of the body surface, without liver or gut involvement, should be treated or not. In three prospective trials of first line treatment , also patients with grade I acute GvHD were enrolled **(1-3) :** however most Centers would probably treat only GvHD grade II or more**.** One argument in favour of steroid treatment, would be early intervention, thus possibly preventing progression to more severe GvHD: this is a general rule of medicine, but evidence that this is the case also in patients with acute GvHD, is lacking **(1,2).** In one randomized study , the Gruppo Italiano Trapianti di Midollo Osseo (GITMO)

had shown back in 1998, that early intervention with high dose steroid (10 mg/kg) , was equally effective as a conventional dose of steroids (2 mg/kg) in first line treatment of acute GvHD **(3);** in that study, the proportion of patient who progressed to grade III-IV was similar in the two groups, despite a median interval between transplant and treatment of 12 days, an argument against the hypothesis that early aggressive intervention would be more effective than standard therapy, and would be able to modify the natural course of the disease **(3).** Similar results were seen in a more recent prospective randomized trial , comparing again two different doses of steroids as first line treatment, and again showing no difference in the rate of progression to severe GvHD **(4)**. In addition steroids cause immune deficiency and promote infectious complications **(5).**

On the other hand, early treatment of GvHD could be beneficial: in a retrospective study of unrelated donor transplants in two different Centers , non relapse mortality (NRM) was lower in one Center using anti-thymocyte globulin (ATG) for GvHD prophylaxis and steroid treatment of grade I acute GvHD **(6) .**

Anyhow, whether GvHD grade I should be treated or not, has not been tested in a prospective trial, and GITMO decided to run such trial: the issue was how to calculate the lowest and highest success rate. We used data from the previous GITMO study **(2)**: 25% of patients with grade I GvHD, treated with 6methylprednisolone (6MPred) 2 mg/kg, progressed to grade II-IV GvHD . We hypothesized that patients left untreated would have twice the risk of progression to grade II-IV GvHD, and 170 patients were needed to test this hypothesis.

We are now reporting the results of this trial in patients with grade I GvHD , randomized to receive steroid treatment or no treatment.

METHODS

Study design. This is a Gruppo Italiano Trapianto di Midollo Osseo (GITMO) study, the study name is RAMP08, registered as EUDTRACT N 2008-000413-29. The study has been conducted according Good clinical practice, (GCP) and following the Helsinky declaration. The study protocol was approved by all local Ethical Committees. Data entry was made by eletctronic CRF provided by Clinical Research Technolgy (CRT), Naples, Italy. The study is an open label multicenter , phase 3 randomized study comparing no treatment, versus treatment with 6-methylprednisolone (6MPred) 1 mg/kg per day, for transplanted patients with grade I aGvHD according to Gucksberg's criteria **(7).** Randomization occurred centrally via web, in a 1:1 ratio. Randomization was performed ,

using a dynamic randomization algorithm- with minimization of differences between arm A and B, to no more than 2 patients overall and 3 patients per strata. Stratification was done by phase (early/advanced) and donor type (matched sibling / alternative donors).We appliead a modified intention to treat analysis, and all patients with at least 1 day of follow up were analyzed in the arm to which they had been allocated: 173 patients were randomized , and 171 were analyzed.The study outline is shown in **Figure 1:** Patients randomized to *observation*, were left untreated. Patients progressing to GvHD II-IV were considered to have reached the primary end point of the study, independent of the interval from randomization, and were treated according to standard procedures of each Center. Patients randomized to *observation* and not progressing were followed up. Patients randomized to *treatment* received 6MPred 1mg/kg/day for 5 days; Patients progressing to GvHD II-IV, had reached the primary end point of the sudy and were treated as per Center policies. If GvHD did not progress , 6MPred would be tapered as follows: 0.75 mg/kg/day on days 6-10, 0.5 mg/kg/day , on days 11-15 , 0,25 mg/kg/day days 16-20, 0,12 mg/kg /day on days 21-30, and discontinued on day +30.

Endpoints. The primary epoind point was the cumulative incidence of patients progressing to grade II-IV aGvHD. Secondary endpoints were : proportion of patients with GvHD III-IV, proportion of bacterial infections, viral infections , fungal infections; number of adverse events and severe adverse events ; cumulative incidence of non relapse mortality (NRM) , cumulative incidence of relapse, proportion of patients developing chronic GvHD (limited and extensive), actuarial overall survival (OS).

Centers . The following Centers participated in the trial: Genova Ospedale San Martino, (A BAcigalupo) Catania Ospedale Ferrarotto (G Milone) , Roma Ospedale San Camillo (A Locasciulli) , Pescara , Ospedale Civile (A Santarone), Torino, Ospedale Regina Margherita (F Fagioli) , Roma Universita' Cattolica (S Sica, P Chiusolo) , Cuneo Ospedale (N Mordini), Alessandria Ospedale Civile (R Sorasio).

Inclusion and exclusion criteria

Eligible for this study were patients aged 0-70, having received an allogeneic stem cell transplant , for malignant or non malignant diseases, developing a skin rash over 10-49% of the body surface , within the previous 48 hours; patients had received an unmanipulated graft from any donor type, and had not received previous treatment with steroids . Signed informed consent was obtained from adults, or tutors of children. Conventional GvHD prophylaxis was given to all patients with cyclosporin methotrexate , with the addition of ATG for unrelated donors, and post-transplant cyclophosphamide (PT-CY) for the small

number (n=15) of HAPLO grafts. A skin biopsy , was recommended but not mandatory; centralized histopathology was provided (D.Massi Firenze).

Exclusion criteria were life threatening infections, evidence of hematologic relapse, investigational drugs for GvHD prophylaxis, patients on steroid treatment (>0.5 mg/kg for 48 hours), grade II-IV GvHD. Progression to gut GvHD, but not liver GvHD, was confirmed by histology.

Randomization. 173 patients with grade I GvHD , grafted in 8 GITMO Centers, were randomized between july, 7, 2009 and august ,12,2014 to a *treatment* arm (6MPred 1 mg/kg/day i.v. for 5 days, with tapering and discontinuation on day +30, or to an *observation* arm **(Fig.1)**. Patients progressing to grade II-IV acute GvHD, had reached the primary end point, and were treated according to standard procedures in eah Center with 6MPred 2 mg/kg/day.

Patients. The clinical characteristics of the two groups -*observation/treatment* – are outlined in **Table 1**. Patients were well balanced in terms of diagnosis (p=0.7): the most frequent diagnosis was AML (n=75) , followed by ALL (n=39) and MDS (n=12). The median age for *observation / treatment* was 46 years (1-69) vs 38 years (0,4-68), (p=0.06); the proportion of patients over 50 years was 51% in the *observation* and 49% in *treatment* group (p=0.8). The donor type in the *observation /treatment* arms was as follows : HLA identical siblings n=36/n=34, unrelated cord blood (CB) n=7/n=1, unrelated donor (UD) n=36/n=44, and haploidentical family donors (HAPLO) 6/7 (p=0.1). The proportion of 1 antigen mismatched unrelated donors was respectively 7 and 9 (p=0.9). Disease phase was classified as early, in 43 *observation* and 38 *treatment* patients (p=0.7). The conditioning regimen was myeloablative in most patients (64 and 61, p=0.8).

Supportive care

Antibacterial prophylaxis with quinolones was given during the neutropenic phase. All Centers used PJV prophylaxis with cotrimoxazole and monitored cytomegalovirus (CMV) reactivation by PCR or antigenemia twice weekly; pre-emptive therapy was instituted in case of CMV reactivation ; Epstein Barr Virus (EBV) was monitored by PCR weekly and treated pre-emptively, if positive. Aspergillus antigenemia with galactomannan was also monitored weekly: diagnosis of invasive fungal disease was performed by standard criteria and treated accordingly. Specific infectious disease policies were performed according to each Centers standard procedures..

Statistical analysis.

The analysis of the primary end point was performed using the cumulative incidence (CI) of GvHD grade II-IV, which was calculated with mortality due to any cause as a competing

risk; non relapse mortality (NRM) was the competing risk for relapse related death (RRD) and viceversa. The Gray's test was used to calculate difference between CI curves. Survival was calculated with Kaplan Meier curves, and the log rank test was used to test for difference between survival curves. The Cox test was used for multivariate analysis. Chi square tables, Fisher exact test, two sample T test, were used as appropriate. NCSS10 for windows , was used for these statistical analyses.

Infections and adverse events in the two arms, within day 100 from randomization, were assessed using Poisson or Negative Binomial (NB) regression model. Each infection type was considered as a single dependent variable and the decision on whether to use the Poisson or the NB model, was based each time on a Likelihood-ratio test for overdispersion of the dependent variable considered. The treatment group indicator was considered as independent variable and the likelihood-ratio test was used to test the association with infections. The total follow-up of each patient was considered as an exposure variable into the model. Stata (v.14) was used for the computation.

Sample size calculation was made using data from a previous GITMO study **(2)**: 25% of patients with grade I GvHD treated with 6methylprednisolone (6MPred) 2 mg/kg, progressed to grade II or greater GvHD . We hypothesized that patients left untreated would have twice the risk of progression to grade II-IV GvHD, 170 patients were needed to reject the null hypotesis with a power of 90%.

RESULTS

Primary end point and GvHD. The cumulative incidence (CI) of acute GvHD grade II, was 50% in *observation* and 33% in *treatment* patients (p=0.005) **(Fig.2)** . This difference was more pronounced in sibling donor grafts (SIB) (61% vs 32%, p=0.01), as compared to alternative donor grafts (ALT) (42% vs 33%, p=0.1). For patients who progressed, the interval between randomization and progression was significantly shorter in the *observation* arm as compared to the treatment arm (3 vs 9 days, p=0.03, **Table 2) . Figure 3,** outlines the CI of GvHD grade III-IV in the *observation* vs the *treatment* arm (13% vs 10%, p=0.6); it was seen in 7 vs 4 sibling grafts , and in 4 vs 5 alternative donor grafts, respectively (p=0.8). It was seen more frequently in patients randomized before day 20 from transplant (n=88; 17%) as compared to patients randomized later (n=83, 6%) (p=0.02). This was true irrespective of randomization to *observation* or *treatment*: 18% vs 16% for early GvHD ($\leq$day 20) , and 7% vs 5% , for late GvHD day 20). Moderate/severe

chronic GvHD was comparable , and diagnosed in 10 *observation* vs 15 *treatment* patients (p= 0.3).

Greatest severity of GvHD , beyond grade I . Skin GvHD , stage 3,4 was diagnosed respectively in 22, 1 *observation* vs 15, 2 *treatment* patients; liver GvHD stage 1,2, 3 , was diagnosed in 4, 3, 1 *observation* vs 7,0,0 *treatment* patients; gut GvHD stage 1, 2, 3, 4, was diagnosed respectively in 10, 3, 3, 1 *observation* vs 3, 2, 3, 1 *treatment* patients.

Steroid dose and additional treatment.

The median cumulative dose of 6MPr received in the first 100 days was 9.5 mg/kg (range 0-105) and 24 mg/kg (range 13-180) in the *observation* and *treatment* groups respectively (p=0.01) **(Table 2)**. Of the 86 patients in the *treatment* arm 57 (66%) were off steroids by day +30 , whereas 29 were on steroids having progressed to grade II-IV GvHD. Of the 85 patients in the *observation* arm , 41 (48%) never received steroids. The use of a second immunosuppressive drug for GvHD, in addition to corticosteroids, was reported in 27 *observation* and 17 *treatment* patients (p=0.08); a third drug in 12 and 7 patients (p=0.2) and a fourth drug in 4 and 2 patients repectively (p=0.4). The second drug included mycophenolate mophetil (MMF) (respectively 6 and 7 patients), extracorporeal photopheresis (ECP) (12 and 4 patients), or infliximab, etanercept,0 rituximab, basiliximab , sirolimus, anti-CD26 antibody , in a few patients each. The third added drug included ECP (3 and 2 patients), MMF (5 and 1), etanercept or anti-CD26 antibody. The fourth added drug included MMF and ECP.

Infections and adverse events.

Table 3 summarizes the adverse events in the two randomization arms in the first 3 months of treatment : the *observation* arm had less bacterial, fungal and CMV infections as compared to the *treatment arm*; other adverse events and other severe adverse events, were also fewer in the *observation* arm **(Table 3),** although man not statistically significant**.** Other adverse events were reported as 17 and 33 respectively in the two arms **(**p=0.11), of which 3 and 11 respectively were classified as severe (p=0.07). The adverse events included steroid associated diabetes (0 vs 9 in *observation* and *treatment* arm respectively), acute renal failure (2 vs 2) cystitis (5 vs 5) , hip necrosis (0 vs 2), multi organ failure (0 vs 2), respiratory failure (0 vs 3), thrombosis (0 vs 2). Median blood counts were comparable on day +60 from randomization, in the *observation* and *treatment* arm respectively. On day +60, chemistry results in the *observation* and *treatment* arm , were also comparable.

Non relapse mortality.

The 5 year CI of non relapse mortality (NRM) was 20% (*observation*) vs 26% (*treatment*) (p=0.2) **(Fig.4)**, and was comparable also after stratifying patients for age ≤40 years (12% vs 19%) and >40 years (28% vs 34%) . In univariate analysis, there was a very strong influence of the interval between transplant and randomization, on NRM, the median interval being 20 days: the cumulative incidence of NRM was 31% vs 18% (p=0.0006) for patients randomized before or after day +20 from transplant. For early randomization (≤ 20 days from transplant) NRM was 24% vs 46% (p=0.02) in *observation* versus *treatment* patients, due to an excess of infections in the *treatment arm* (2 vs 8); for late randomization (>20 days) NRM was comparable in the two arms (22% and 14% ; p=0.3).

Relapse related death and survival. Relapse related death (RRD) was 25% in *observation* vs 21% in *treatment* patients **(Fig.5):** patients with early disease had a significant lower probability of RRD in univariate analysis (RR 0.3, p=0.006)**.** RRD was unaffected by the interval between transplant and randomization. Actuarial 5 year survival was 51% (*observation*) vs 41% (*treatment*) (p=0.3)**(Fig.6)** . Predictors of survival in univariate analysis, were younger age , early disease phase and randomization beyond day +20 from transplant. Causes of death in the *observation/treatment* groups were as follows (**Table 2)**: GvHD in 9 vs 13 patients ; infection in 7 vs 9; toxicity , in 1 patient in each group, leukemia relapse in 20 vs 16 patients (p=0.9). There was no significant difference in NRM among different Centers (p=0.5).

Skin biopsies. A skin biopsy to prove or disprove skin GvHD, was not mandatory for eligibility in this trial: it was performed before randomization and reviewed centrally by one of the Authors (DM) in 38 patients: of these 36 (95%) were compatible with acute GvHD (proven , probable and possible respectively in 9,15 and 12 patients respectively).These diagnoses were equally distributed in *treatment* and observation patients (p=0.7) .

Multivariate analysis. Progression to GvHD II-IV was predicted in a Cox analysis, by age older than 20 (p=0.003) or 40 years (p=0.005), and being assigned to the *observation* group (p=0.02) **(Table 4)**. A short interval between transplant and randomization (≤ 20 days), was the only variable predicting progression to GvHD grade III-IV (RR 0.4, 95%CI 0.12-0.98; p=0.04) and was also associated with a higher risk of death (p=0.006) **(Table 4).** Survival was also predicted by patients age and disease phase. Non relapse mortality was predicted only by age > 20 years (RR 2.8, 95%CI 0.88-9.18, p=0.07) and age >40 years (RR 3.0, 95%CI 0.99-9.67, p=0.051, and by early onset of GvHD < day +20 from transplant (RR 0.4 95%CI 0.25-0.94, p=0.03).

DISCUSSION

Treatment of acute GvHD remains a difficult issue, despite several decades of studies and many immunosuppressive/ immunomodulating agents tested **(8).** Difficulties reside not only in the treatment, but they start with staging of involved organs and overall grading of the disease**,** with several possible grading options , and a degree of variability, according to the assessor **(7-13).** Despite the grading variability, and the difficulty in assessing response rates, it is recognized that mortality increases with increasing GvHD severity, and this is true both in the short and in the long term **(14):** in a large group of patients (n=4174) NRM at 3 years, was 21% for grade 0 - I acute GvHD , 32% for grade II, 60% for grade III and 89% for grade IV; the corresponding overall survival at 3 years was respectively 79%, 64%, 37% and 10% **(14).** This study exemplifies on one hand, the major impact of acute GvHD grading on the outcome of allogeneic transplants, and on the other, the lack of effective treatment, when the disease is beyond grade II. In keeping with the latter obervation, a recently developed risk score for acute GvHD, identifies patients at high risk of mortality, according to the number of involved organs and the severity of GvHD at onset **(13):** the mortality at 6 months is 22% for standard risk and 44% for high risk GvHD (**13).** A set of biomarkers of GvHD have recently been described, and identify, at the onset of the disease, severe cases with high mortality, eligible for early intervention **(15).**

It would thus seem reasonable to try and prevent progression of acute GvHD, and this may be achieved if acute GvHD is treated at a very early stage, the earliest being grade I, or a skin rash involving less than 50% of the body surface. We therefore asked whether steroid treatment of grade I GvHD**,** would be beneficial, and we selected evolution to grade II or more, as the primary end point of the study. Patients randomized in the *observation* arm, would become eligible for treatment when diagnosed as GvHD grade II, also if this occurred 24 hours after randomization: this made the informed consent easy to discuss with the patients, since there would be no delay in treatment, once the disease had progressed to grade II. As expected, patients randomized to receive *treatment*, at diagnosis of grade I GvHD, had a significantly lower probability to progress to grade II or more GvHD , when compared to untreated patients (33% vs 50%). The fact that *observation* patients, grafted from identical siblings, had a higher proportion of grades II-IV GvHD (61%) as compared to *observation* patients receiving alternative donor grafts (44%), can be explained by the fact that in the latter, GvHD prophylaxis included either ATG or PT-CY, in addition to CyA and MTX (UD grafts) or CyA and mycophenolate (HAPLO grafts). The unexpected finding was that the cumulative incidence of patients progressing to severe (grade III-IV) GvHD, was comparable in the two groups (13% vs 10%) .

Therefore, the primary end point of the study was reached, but this was due to skin GvHD progressing from stage 2 to stage 3 in the *observation* arm (52 *observation* vs 21 *treatment* patients) and stage 1 gut GvHD (10 *observation* vs 3 *treatment* patients). On the other hand patients with stage 2-4 gut GvHD were comparable in the two randomization groups (7 and 6) , and liver GvHD was seen in a few patients only.

When looking at adverse events , we found that patients in the *treatment* arm, had more infectins and more adverse events than *observation* patients: in particular, bacterial infections, severe fungal infections and CMV reactivation. As a consequence of similar severe GvHD and more infections, non relapse mortality was 20% in the *observation* versus 26% in the *treatment* arm, and survival at 5 years was respectively 51% versus 41% : in a multivariate Cox analysis , there was a trend for inferior survival (p=0.09) in the *treatment* arm, despite a median younger age (38 vs 46 years).

Other studies have tested early treatment of GvHD (**2-4).** Etanercept and topical steroids have been reported in grade I GvHD **(16):** 34 patients entered that prospective study: the proportion progressing to grade III-IV was 3% , significantly lower compared to a contemporaneous group of patients receiving topical steroids alone , 18% of whom progressed to grade III-IV GvHD **(16)**. Although that study suggested that etanercept was able to modify the natural course of the disease, NRM at 2 years was 19% **(16)**, which seems comparable to the 20% NRM of our *observation* group, and the 26% of our *treatment* group. An other non steroid approach was tested propectively, randomizing patients to receive or not to receive 2.5 mg/kg of anti-thymocyte globulin (ATG) , on day +7 after an alternative donor transplant **(17):** GvHD III-IV was significantly reduced in the ATG group (5%) as compared to the untreated group (15%), though NRM was only marginally reduced from 35% to 29%(p=ns) **(17).** Finally high dose cyclophosphamide post transplant, is being widely and successfully used to prevent severe GvHD **(18-20),** but again this is given very early, on day +3 , and possibly interfering with the activation phase of T cells, rather than with the effector phase.

We found a strong association of early GvHD, with GvhD severity and survival : patients developing grade I GvHD within day +20 from transplant, had a higher probability (RR 2.7) of developing gade III-IV GvHD, as compared to patients randomized later (17% versus 6%, p=0.02), and a higher risk of NRM (31% vs 18%; p=0.0006). Randomization to steroids was not beneficial in these early grade I GvHD patients, with progression to grades III-IV in 18% *observation* versus 16% *treatment* patients. In addition there was an excess of infectious mortality in patients randomized $\leq$ day 20 to the *treatment* arm.

Also survival was predicted by the time of randomization, in univariate and multivariate analysis: the 4 year survival of patients randomized before day +20 from transplant was 33%, compared to 60% for patients randomized later (p=0.001), and this was true both if assigned to *observation* or to *treatment*. In our data base of 2445 allogeneic transplants, the proportion of grades III-IV GvHD in patients develping GvHD within day 20, between day 21-40 or beyond day 40, is 11%, 9%, 3% (p=0.0002) , and NRM is respectively 35%, 28%, 25% (p=0.0006) (unpublished), confirming other reports on the association of early onset as a risk factor for grade III-IV GvHD **(21)**.

In conclusion, steroid treatment of GvHD grade I prevents progression to grade II GvHD , but not to grade III-IV GvHD , and there is no beneficial effect on NRM and survival. In addition patients receiving steroids are at a higher risk of developing infections, and have more adverse events. especially if GvHD develops within day +20 from transplant, A small proportion of patients develop life threatining GvHD, irrespective of early steroid treatment , suggesting that the severity of GvHD is set at onset. Early identification of high risk patients, with recently described biomarkers **(23)**, and pre-emptive treatment with non steroidal agents , should be investigated, with the aim of changing the natural course of the disease.

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Authorship . AB, AR and CS designed the study ; AB, GM, AP, FF, MB, AS, PC, SS, SM, RS, MTVL, AMR, AR contributed patients , DM provided histologic evaluation of skin biopsies; MPS and AS ran the statistical analysis; AB, CS, FB wrote the manuscript, and all authors discussed the results and approved manuscript. The Authors declare no conflict of interest.

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Table 1. Clinical data of patients randomized

	Observation	**Treatment**	**P**
N=	85	86	
Age	46 (1-69)	38 (0.4-68)	0.06
Gender M/F	37/48	35/51	0.7
Diagnosis			
SAA	2	2	0.7
AML	41	34	
ALL	16	23	
CML	3	1	
MDS	5	7	
Myelofibrosis	4	5	
Myeloma	3	6	
CLL	3	2	
NHL	4	3	
HD	2	1	
Other	2	2	
Disease phase:early	43 (53%)	38 (47%)	0.7
Donor type			
Matched SIBS	36 (42,4%	34 (39,5)	0.1
UD	36 (42,4%	44 (51,2%)	
HAPLO	6 (7,1%)	7 (8,1%)	
CB	7 (8,2%)	1 (1,2%)	
Conditioning MA/RIC	64/21	61/25	0.8

Abbreviations: M/F= male female; SAA= severe aplastic anemia; AML= acute myeloid leukemia; ALL= acute lymphoblastic leukemia; CML= chronic myeloid leukemia; MDS= myelodylastic syndromes; CLL= chronic lymphocytic leukemia; NHL= non Hodgkin's lymphoma; HD= hodgkin's disease; UD= unrelated donor; SIBS= siblings; HAPLO= HLA haploidentical donors; CB= cord blood ; MA= myeloablative; RIC= reduced intensity

Table 2. Outcome of patients

	Observation	Treatment	P
N=	85	86	
Interval Transplant-Random (days)	20 (4-120)	20 (3-102)	0.5
Interval Random -GvHD II-IV(days)	3 (0-37)	9 (0-63)	0.03
GvHD II-IV (n.pts)	44	29	0.01
GvHD III-IV (n.pts)	11	9	0.6
Chronic GvHD moderate/severe	10	15	0.3
Steroid dose mg/kg <100 days	9.5 (0-105)	24 (13.5-180)	0.01
Causes of death			
GvHD	9	13	0.5
Infections	7	9	
Toxicity	1	1	
Relapse	20	16	

Table 3. Infectious episodes and adverse events in the two arms <100 days from randomization

	Observation	Treatment	P value*
N=	85	86	
FU days at 100 days	8040	7934	
FU days/patient	94,5	92,2	
Bacterial infections	12	25	0.045
Severe bacterial inf.	3	8	0.16
Lethal bacterial inf.	2	5	0.29
Fungal infections	8	8	0.94
Severe fungal inf.	0	3	0.047
Lethal fungal inf.	0	2	0.11
CMV	63	84	0.48
Sever CMV inf.	3	3	0.94
Lethal CMV infections	0	3	0.046
Other viral infections	24	15	0.32
PTLD	2	1	0.53
Other AE	17	33	0.11
Other severe AE	3	11	0.077
Lethal AE	0	4	0.041

One patient can have more than one infectious episode or adverse event.
=Abbreviations= AE= adverse events; inf= infections; CMV= cytomegalovirus; PTLD= post transplant lymphoproliferative disease.
*P value: Poisson or Negative Binomial (NB) regression model (see statistical methods)

Table 4. Multivariate analysis

			GvHD II-IV			Overall Survival		
	Base	Compared	RR	95% CI	P	RR	95%CI	P
Age (yy)	**<20**	**>20**	**3.6**	**1.03-12.8**	**0.04**	**2.4**	**1.09-5.58**	**0.02**
		>40	**5.6**	**1.71-18.6**	**0.004**	**2.9**	**1.28-6.13**	**0.009**
Gender	M	F	0.9	0.8		0.9		0.9
Donor	ALT	SIB	1.2	0.4		0.8		0.4
Phase	ADV	EARLY	0.6	0.1		**0.5**	**0.35-0.9**	**0.02**
Dx	AL	Other	0.9	0.8		1.1		0.6
SC source	BM	PB	0.7	0.3		0.9		0.8
		CB	0.4	0.4		0.4		0.3
Rand	**OBS**	**TREAT**	**0.5**	**0.35-0.99**	**0.04**	1.4		0.1
Int.Tx-Rand	**≤20**	**>20 dd**	0.7	0.2		**0.4**	**0.26-0.71**	**0.001**

Abbreviations: Base= baseline value; Compared= compared value; RR=relative risk; P= p value; GvHD graft versus host disease; Rand= randomization group; OBS= observation; TREAT= treatment; Dx= diagnosis; AL= acute leukemia. Int Tx-Rand= interval in days (dd) between stem cell transplantation and randomization. Donor: ALT= alternative, SIB= identical sibling

Legend for Figures

Fig.1. Outline of the study. 6MPred =6 mthylprednisolone. Patients randomized to the *observation* (n=85) or *treatment* arm (n=86) were all analyzed . FU= follow up; Two patients were not evaluable because essential data were missing; 1 *observation* and 1 *treatment*

Fig.2. Cumulative incidence of acute graft versus host disease (GvHD) grade II-IV, in patients allocated to no treatment (*observation*s) or treatment with prednisolone 1 mg/kg (*treatment*).

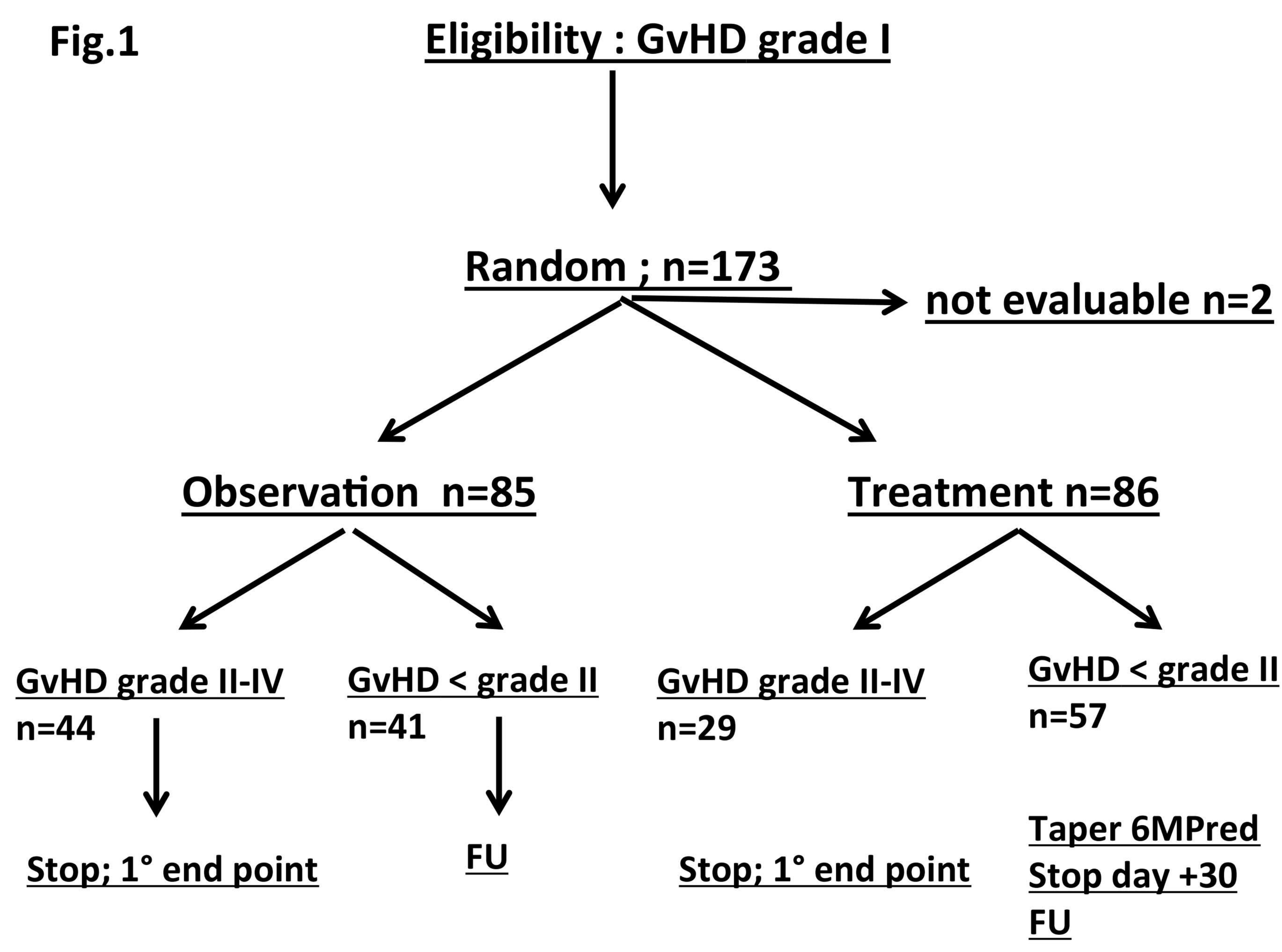
Fig.3 Cumulative incidence of acute graft versus host disease (GvHD) grade III-IV, in patients allocated to no treatment (*observation*s) or treatment with prednisolone 1 mg/kg (*treatment*).

Fig.4 Comparable cumulative incidence of non relapse mortality (NRM) in the two randomization groups.

Fig.5 Comparable cumulative incidence of relapse related death (RRD) in the two randomization groups

Fig.6 Comparable 5 year overall survival in the two randomization groups

Fig.1


Eligibility : GvHD grade I
Random ; n=173
not evaluable n=2
Observation n=85
Treatment n=86
GvHD grade II-IV
n=44
GvHD < grade II
n=41
GvHD grade II-IV
n=29
GvHD < grade II
n=57
Stop; 1° end point
FU
Stop; 1° end point
Taper 6MPred
Stop day +30
FU

Cumulative incidence of acute GvHD grade II+

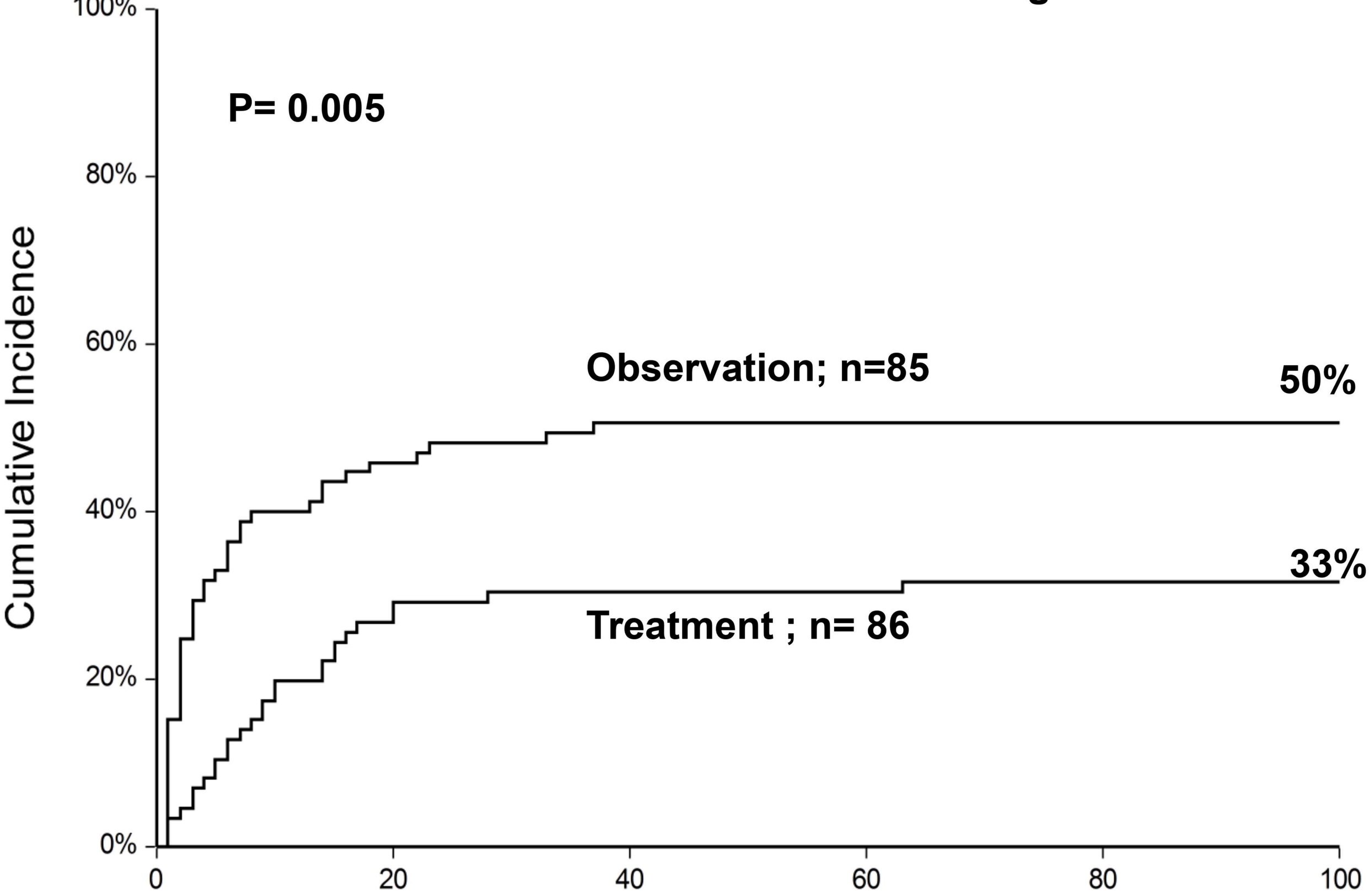


Fig.2

Cumulative incidence of acute GvHD grade III-IV

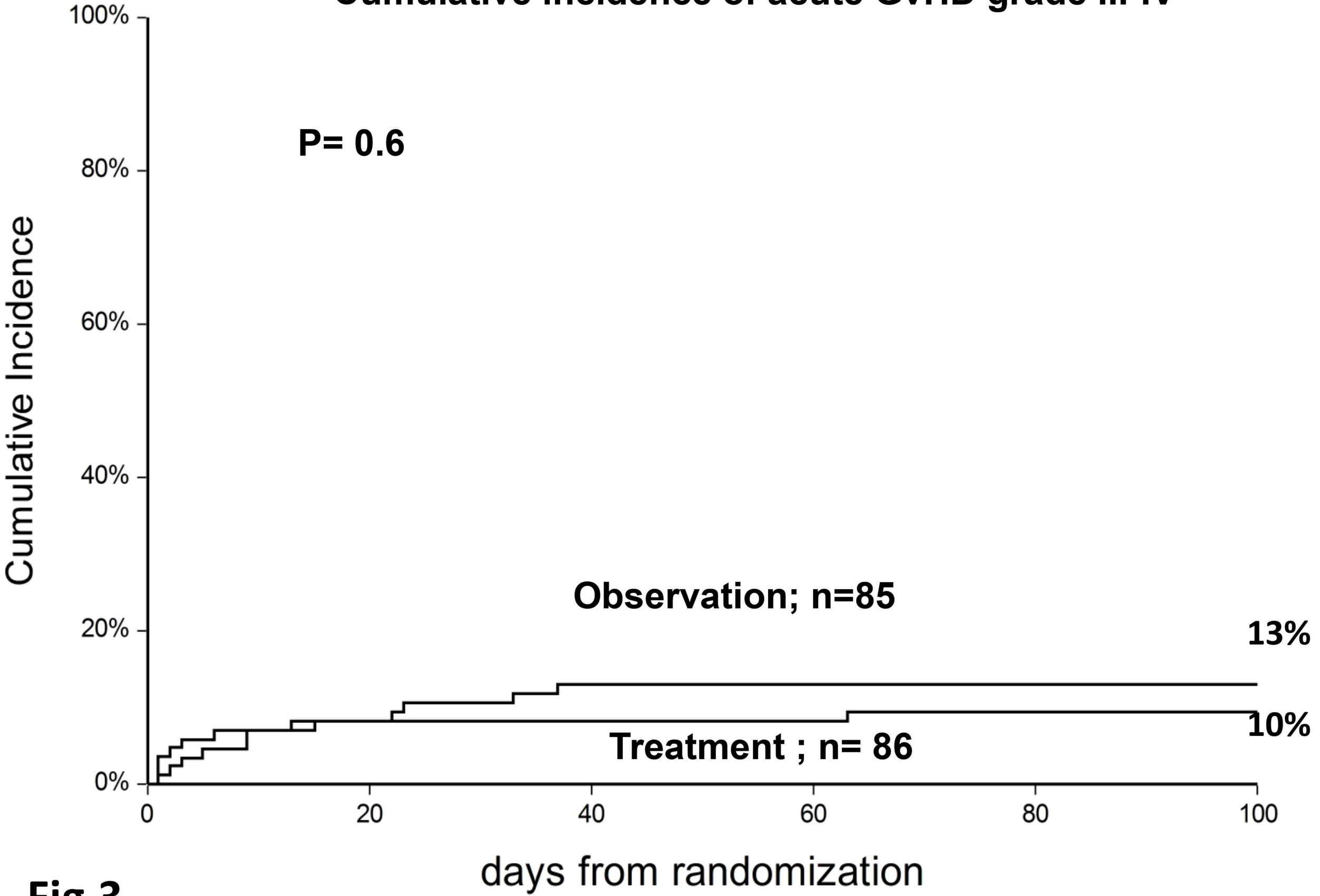


Fig.3

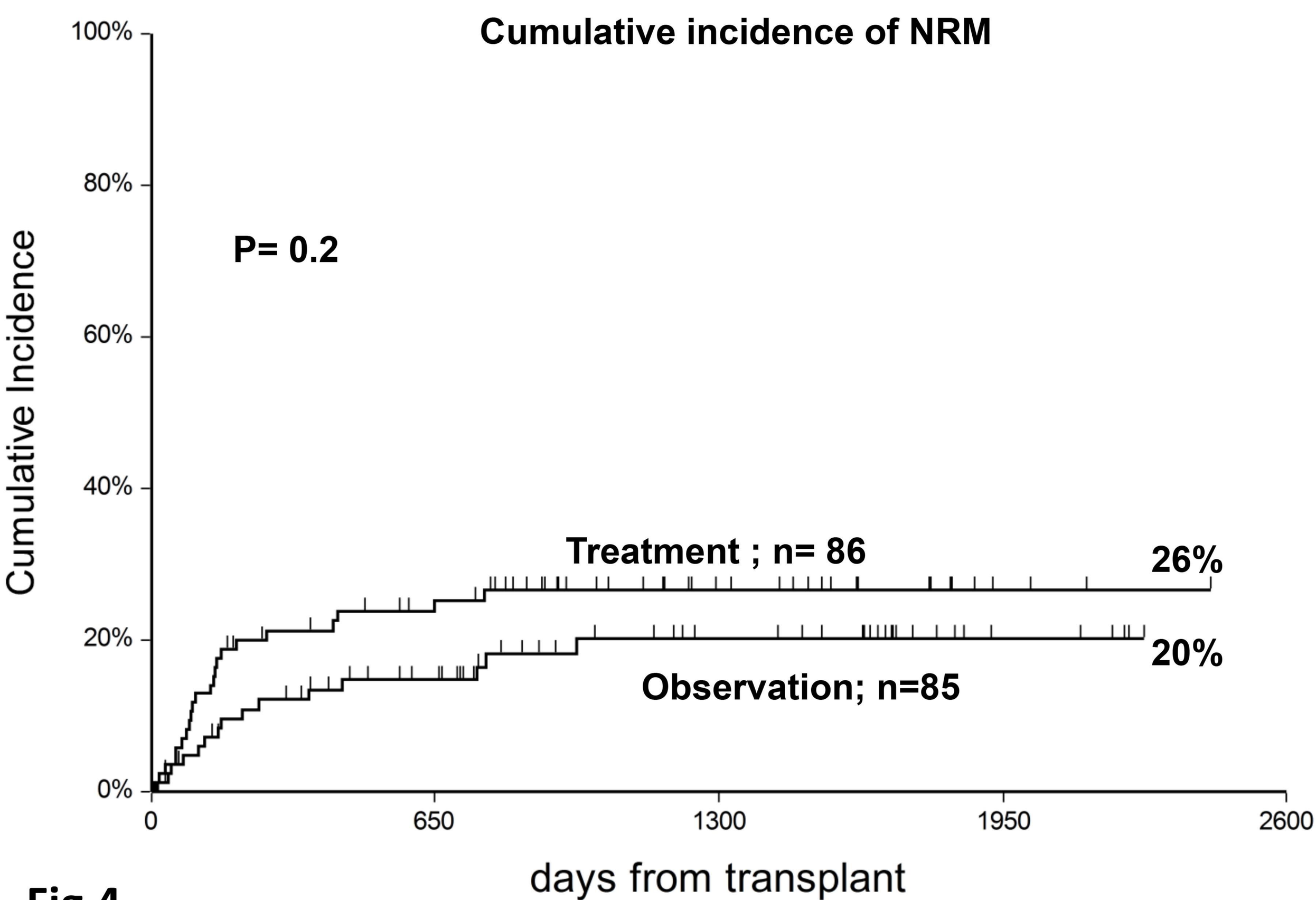

Cumulative incidence of NRM
P= 0.2
Treatment ; n= 86
26%
20%
Observation; n=85
Cumulative Incidence
100%
80%
60%
40%
20%
0%
0
650
1300
1950
2600
days from transplant

Fig.4

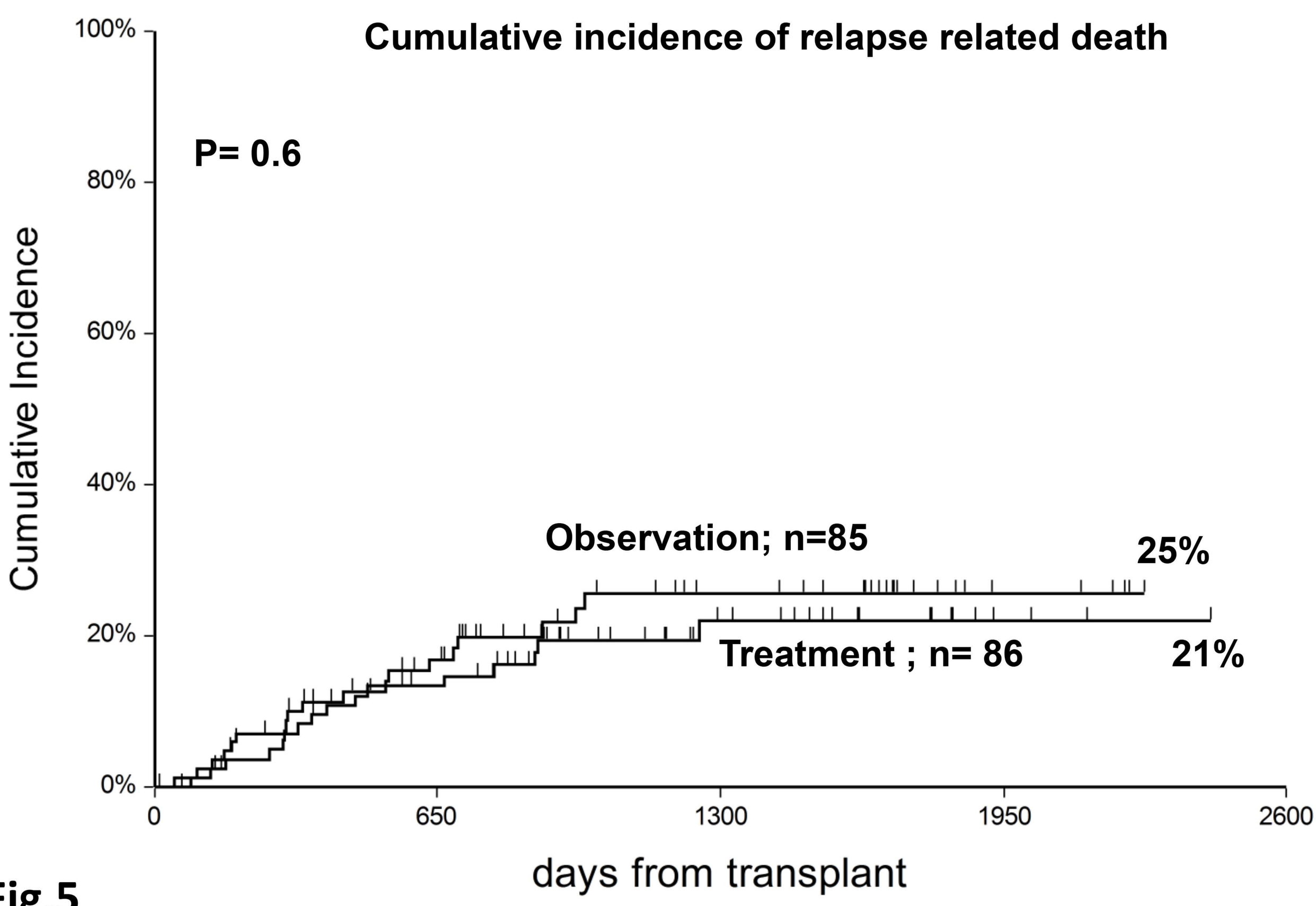

Cumulative incidence of relapse related death
P= 0.6
Observation; n=85
25%
Treatment ; n= 86
21%
Cumulative Incidence
100%
80%
60%
40%
20%
0%
0
650
1300
1950
2600
days from transplant

Fig.5

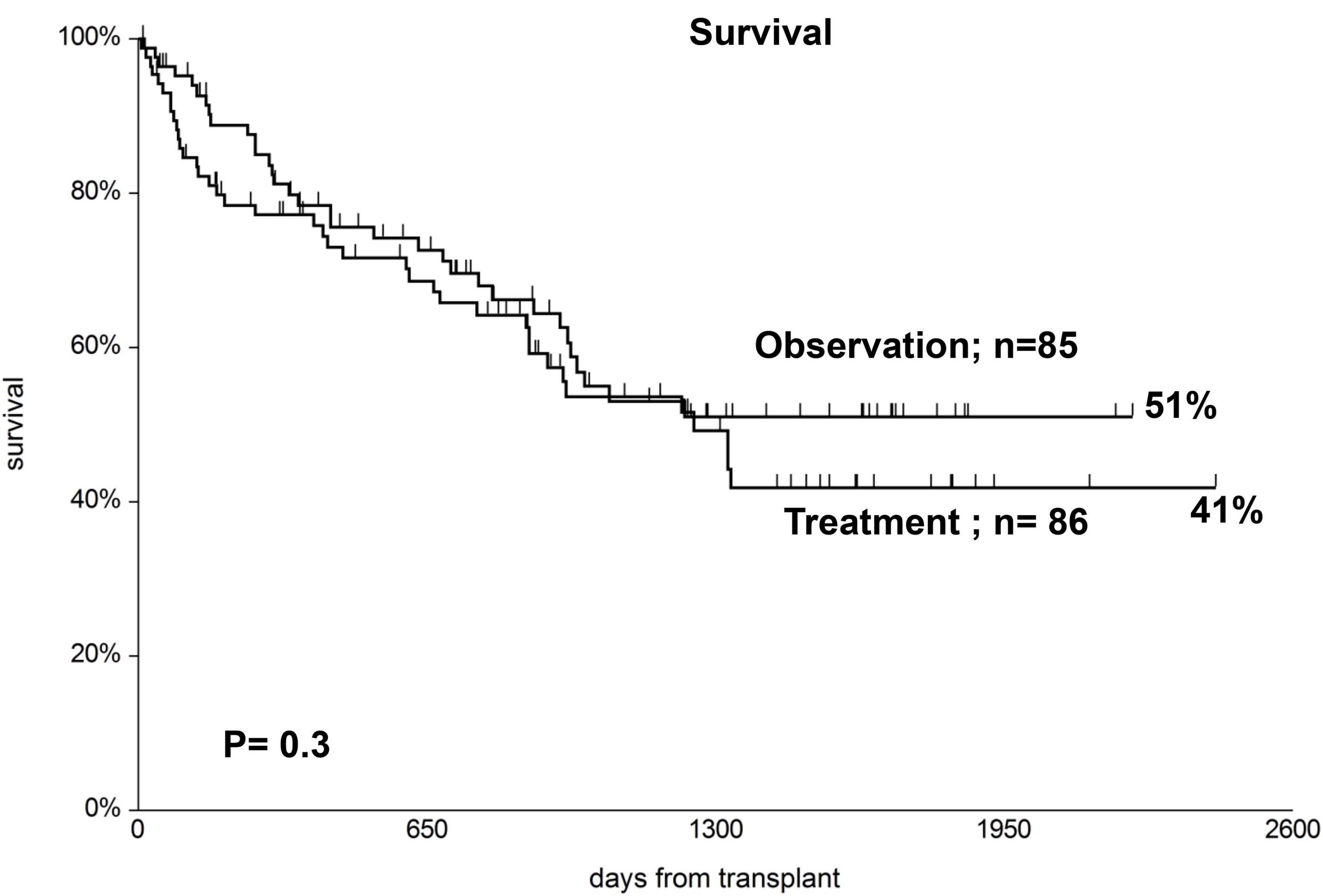

Survival
100%
80%
60%
40%
20%
0%
survival
Observation; n=85
51%
Treatment ; n= 86
41%
P= 0.3
0
650
1300
1950
2600
days from transplant

Fig.6