

# Review of Aplastic Anemia Guidelines



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**Asia-Pacific Blood and Marrow Transplantation Group**

Dear Dr. Kojima

I write to ask for your expert opinion in the management of a difficult case.

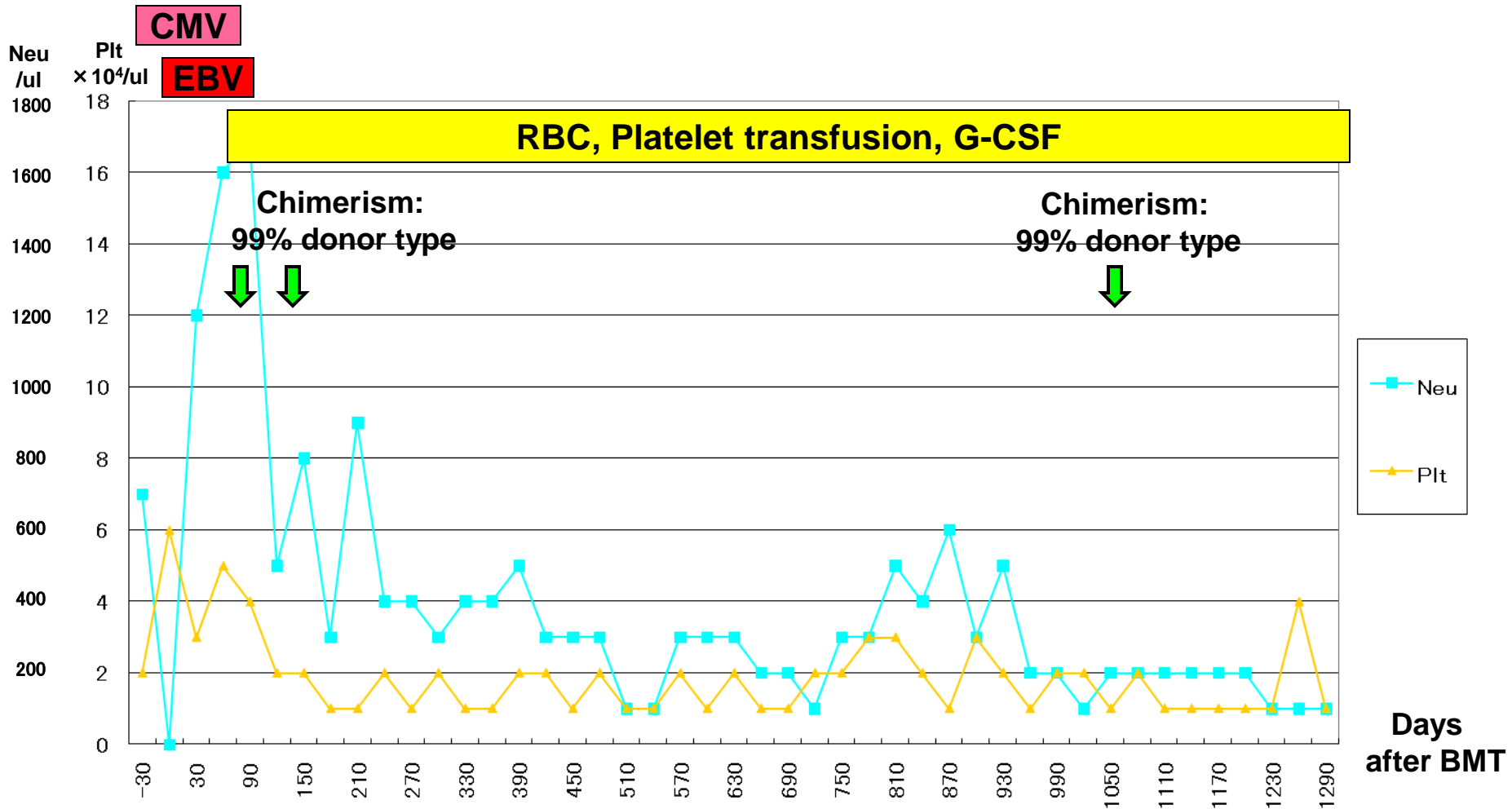
A 14 year-old girl developed aplastic anemia. Two HLA-identical sisters were identified. She received a BMT from the older sister after cyclophosphamide/ATG conditioning. Despite complete donor chimerism her count recovery was transient and she became transfusion-dependent again. A number of immunosuppressive agents were tried, without response. She was also given DLI x1, mobilized stem cell boost twice from the same donor without improvement.

The patient then received a myeloablative conditioning regimen (Busulfan 16 mg/kg, Thiotepa 10 mg/kg, Fludarabine 160 mg/m<sup>2</sup> and Thymoglobulin 9 mg/kg) and given a marrow graft from the other HLA-identical sibling. Again she showed no hematologic recovery in the presence of donor chimerism.

From Texas

# Clinical course

11yF, RCC, Donor: matched unrelated,  
Conditioning regimen: FLU+CY+Campath+TBI(3Gy),  
GVHD prophylaxis: FK506, Cell dose:  $1.5 \times 10^8/\text{kg}$ , aGVHD: grade 1,  
CMV antigenemia (+), EBV-LPD (+), Onset of aplasia after BMT: day110



**Donor-type Bone Marrow Failure!**

# Patients and methods

To clarify risk factors of donor type bone marrow failure, we reviewed clinical data of 58 patients with SAA who received allogeneic bone marrow transplantation (BMT) at Nagoya University Hospital or Japanese Red Cross Nagoya 1<sup>st</sup> Hospital from January 1997 to December 2010.

## Definition of Donor-type Bone Marrow Failure

Persistent cytopenia (Hb<8g/dl, Neu<1,000/ul, Plt<50,000/ul) for >6 months after achieving engraftment with full donor type cells.

# Patients characteristics

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<b>No. of patients</b>	<b>58</b>
<b>Age, median (range)</b>	<b>10 (1-20)</b>
<b>Male / Female</b>	<b>36 / 22</b>
<b>Idiopathic / Hepatitis</b>	<b>54 / 4</b>
<b>Morphological criteria(AA / RCC)</b>	<b>26 / 32</b>
<b>Conditioning regimen</b>	
<b>CY+ATG+TBI</b>	<b>29</b>
<b>CY+ATG</b>	<b>9</b>
<b>CY+TLI</b>	<b>2</b>
<b>Flu+CY+TLI</b>	<b>8</b>
<b>Flu+CY+Campath/ATG+TBI</b>	<b>10</b>
<b>Related donor (n = 25)</b>	<b>HLA-match 20 / mismatch 5</b>
<b>Unrelated donor (n = 33)</b>	<b>HLA-match 14 / mismatch 19</b>

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# Results

No.	Age at BMT	Sex	Year at BMT	Period from Dx to BMT (d)	Morphological classification	IST	RBC transfusion times
1	4	M	2003	460	RCC	—	1
2	11	F	2003	576	RCC	—	0
3	13	M	2005	3164	RCC	+	>100
4	5	M	2005	584	RCC	+	12
5	6	M	2005	373	RCC	—	1
6	11	M	2006	42	RCC	—	0
7	9	M	2006	889	RCC	+	42
8	13	M	2007	3290	RCC	—	0
9	11	F	2008	268	RCC	+	17
10	5	F	2009	266	RCC	+	5
11	2	M	2010	308	RCC	+	20

# Donor status - Conditioning regimen - Complications

No.	HLA	Related / Unrelated	Conditioning regimen	GVHD prophylaxis	Cell dose	ANC >500	aGVHC	cGVHD	Sepsis	CMV GCV	EBV Ritux
1	matched	Relate	FLU+CY+TLI	CyA	6.0	15	—	—	+	+	—
2	matched	Relate	FLU+CY+TLI	CyA	2.5	17	—	—	—	—	—
3	matched	Unrelated	CY+ATG+TBI	FK506	2.0	25	I	—	—	—	—
4	matched	Unrelated	CY+ATG+TBI	FK506	3.1	31	II	extensive	+	+	—
5	matched	Relate	FLU+CY+TLI	CyA	4.9	16	—	—	—	—	—
6	matched	Relate	FLU+CY+TLI	CyA	4.6	16	—	—	—	—	—
7	mismatched	Unrelated	CY+ATG+TBI	FK506	2.0	22	III	—	—	+	+
8	matched	Relate	FLU+CY+TBI	CyA	3.2	16	—	—	—	+	—
9	matched	Unrelated	FLU+CY+Campath+TBI	FK506	1.5	18	I	—	—	+	+
10	mismatched	Relate	FLU+CY+Campath+TBI	FK506	3.5	23	—	limited	—	+	—
11	mismatched	Unrelated	FLU+CY+ATG+TBI	FK506	4.2	24	—	—	+	+	—



# Clinical course

No.	Onset	Period (d)	Hb	Neu	Platelet	recovery	Survival (d)	Chimerism analysis
1	110	1789	5.1g/dl	300/ul	11000/ul	+	3190<	day52: 96% donor (FISH)
2	710	2296<	6.8g/dl	600/ul	31000/ul	—	3026<	day1737 : 100% donor (FISH)
3	161	867	6.4g/dl	100/ul	2000/ul	+	2354<	day90: 98% donor (FISH)
4	46	2275<	4.2g/dl	100/ul	4000/ul	—	2318<	day215:100% donor (STR)
5	240	505	8.9g/dl	700/ul	31000/ul	+	2283<	day68: 95%< donor (STR)
6	370	953	8.0g/dl	200/ul	32000/ul	+	2190<	day72:99.8% donor (FISH)
7	50	460	7.0g/dl	300/ul	10000/ul	+	1982<	day97:100% donor (STR)
8	68	1719<	6.6g/dl	100/ul	11000/ul	—	1807<	day77: 100% donor (STR)
9	110	1194<	6.0g/dl	100/ul	10000/ul	—	1309<	day1062 : 99% donor (FISH)
10	72	732<	5.0g/dl	200/ul	7000/ul	—	802<	day435:95%< donor (STR)
11	107	203	6.0g/dl	600/ul	14000/ul	+	712<	day91: 95%< donor (STR)

# Variables

**Age at BMT**

**Morphological classification**

**Sex**

**Years performed BMT**

**Period from Dx to BMT**

**RBC transfusion times**

**IST before BMT**

**Donor status:**

**Related / Unrelated**

**HLA-matched / mismatched**

**Cell dose**

**Flu regimen**

**Campath**

**TBI**

**CyA / FK506**

**Acute GVHD**

**Chronic GVHD**

**CMV antigenemia**

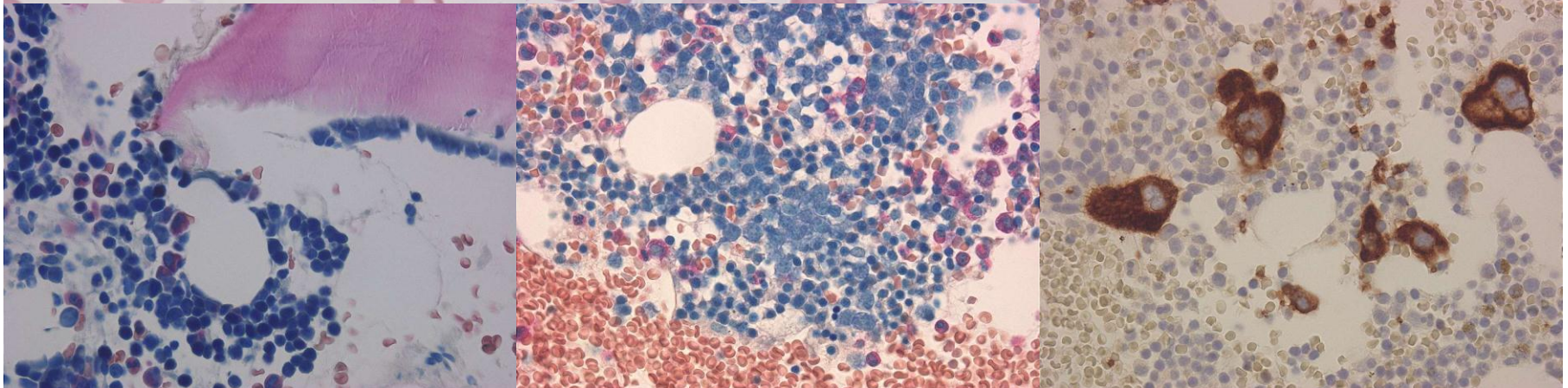
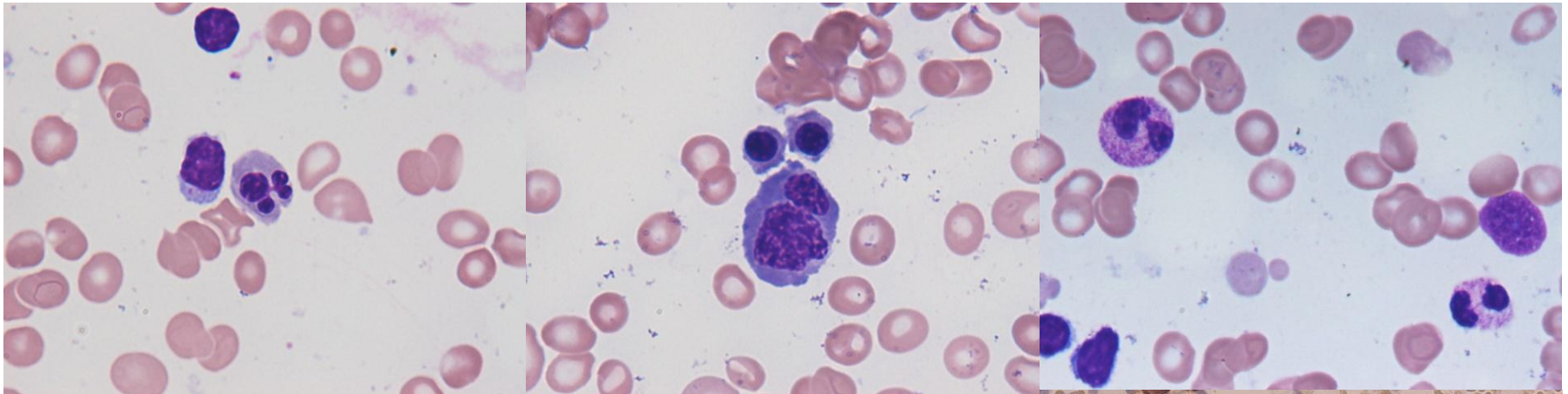
**Ganciclovir**

# Definition of Refractory cytopenia of childhood (RCC)

Blasts <2% in the PB, <5% in the BM  
Dysplastic changes in 2 cell lineages  
or >10% in 1 cell lineage

Nuclear lobulated and bi-nucleated erythroblasts

Hypolobular neutrophils



Abnormal localization and megaloblastic changes of erythroid

CD42b (GpIb) (+) megakaryocytes

# Central review system in Japan

**PB and BM smear**

**Nagoya University  
St. Luke's Hospital**

**Pathology**

**Red Cross  
Nagoya 1<sup>st</sup> Hospital**

**PNH  
Telomere length**

**Nagoya University**

**JMML-gene analysis  
Colony assay**

**Nagoya University  
Shinsyu University**

# Patient characteristics

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**Reviewed (2009.2 – 2011.8)      500 (pathology: 374, 75%)**

**Age, median (range)      6 (0-27)**

**Sex, Male/Female      268 / 232**

## Outcome

**AA / RCC / CBMF      295 (59%)**

**Advanced MDS / AML      44 ( 8%)**

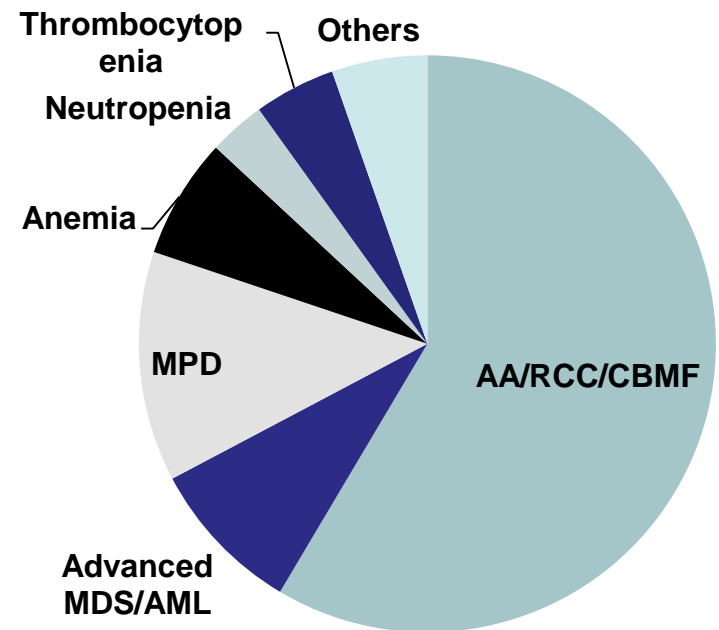
**MPD      65 (13%)**

**Anemia      34 ( 7%)**

**Neutropenia      16 ( 3%)**

**Thrombocytopenia      23 ( 5%)**

**Others      27 ( 5%)**

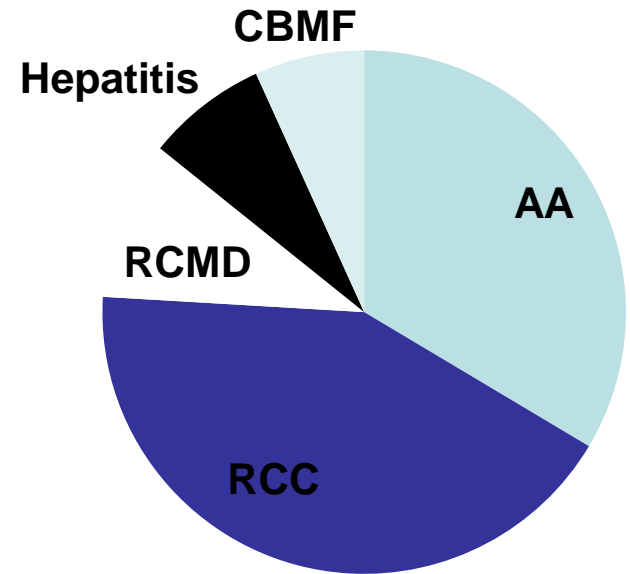


# AA / RCC / CBMF (n = 295)

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AA	99 (33%)
RCC	125 (42%)
RCMD	29 (10%)
Hepatitis related	22 ( 7%)
Fanconi anemia	7 ( 3%)
Dyskeratosis congenita	3 ( 1%)
Shwachman syndrome	5 ( 2%)
CBMF suspected	5 ( 2%)

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<b>Variables</b>	<b>BMF (-)</b>	<b>BMF (+)</b>	<b>p</b>		
No. of patients	47	11			
Age at BMT (median)	10	9	0.835		
Male	28	8			
Female	19	3	0.327		
AA	26	0			
RCC	21	11	0.001		
BMT before 2003	24	2			
BMT after 2004	23	9	0.048		
From Dx to BMT	1000d	>	28	9	
		<	19	2	0.151
RBC transfusion	30 times	>	30	9	
		<	17	2	0.220
IST	(+)		32	6	
	(-)		15	5	0.304
Related donor			18	6	
Unrelated donor			29	5	0.258
HLA-matched donor			23	7	
HLA-mismatched donor			24	4	0.518
Cell dose	$3 \times 10^8/\text{kg}$	>	15	4	
		<	32	7	0.519

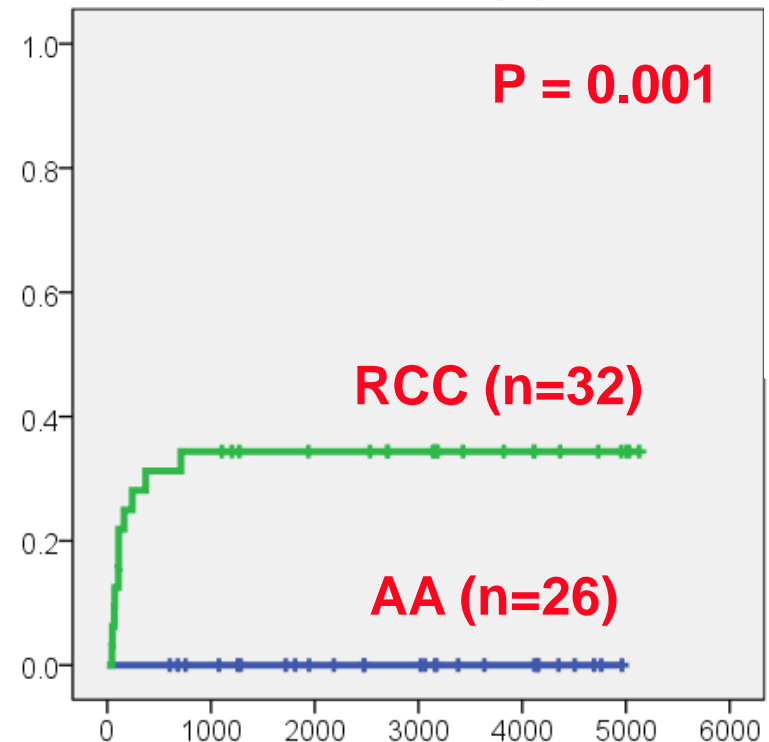
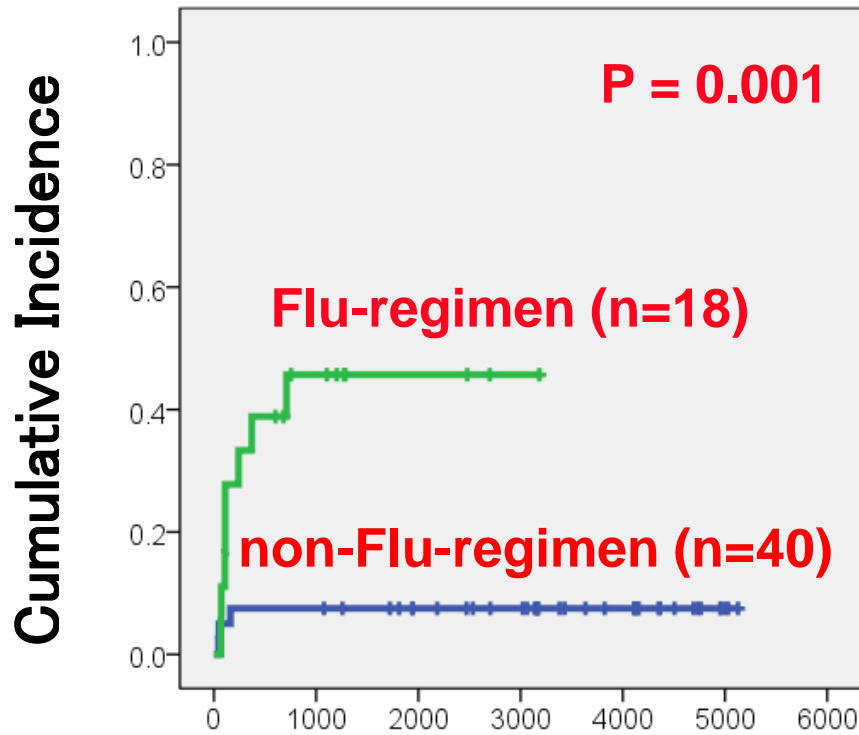
<b>Variables</b>		<b>BMF (—)</b>	<b>BMF (+)</b>	<b>p</b>
<b>No. of patients</b>		<b>47</b>	<b>11</b>	
<b>Flu regimen</b>	<b>(+)</b>	<b>10</b>	<b>8</b>	<b>0.002</b>
	<b>(—)</b>	<b>37</b>	<b>3</b>	
<b>Campath</b>	<b>(+)</b>	<b>7</b>	<b>2</b>	<b>0.547</b>
	<b>(—)</b>	<b>40</b>	<b>9</b>	
<b>TBI</b>	<b>(+)</b>	<b>38</b>	<b>11</b>	<b>0.128</b>
	<b>(—)</b>	<b>9</b>	<b>0</b>	
<b>CyA</b>		<b>21</b>	<b>5</b>	<b>0.611</b>
<b>FK506</b>		<b>26</b>	<b>6</b>	
<b>Acute GVHD</b>	<b>(+)</b>	<b>16</b>	<b>4</b>	<b>0.572</b>
	<b>(—)</b>	<b>31</b>	<b>7</b>	
<b>Chronic GVHD</b>	<b>(+)</b>	<b>12</b>	<b>2</b>	<b>0.470</b>
	<b>(—)</b>	<b>35</b>	<b>9</b>	
<b>CMV antigenemia</b>	<b>(+)</b>	<b>31</b>	<b>7</b>	<b>0.572</b>
	<b>(—)</b>	<b>16</b>	<b>4</b>	
<b>Ganciclovir</b>	<b>(+)</b>	<b>26</b>	<b>7</b>	<b>0.439</b>
	<b>(—)</b>	<b>21</b>	<b>4</b>	



# Multivariate analysis

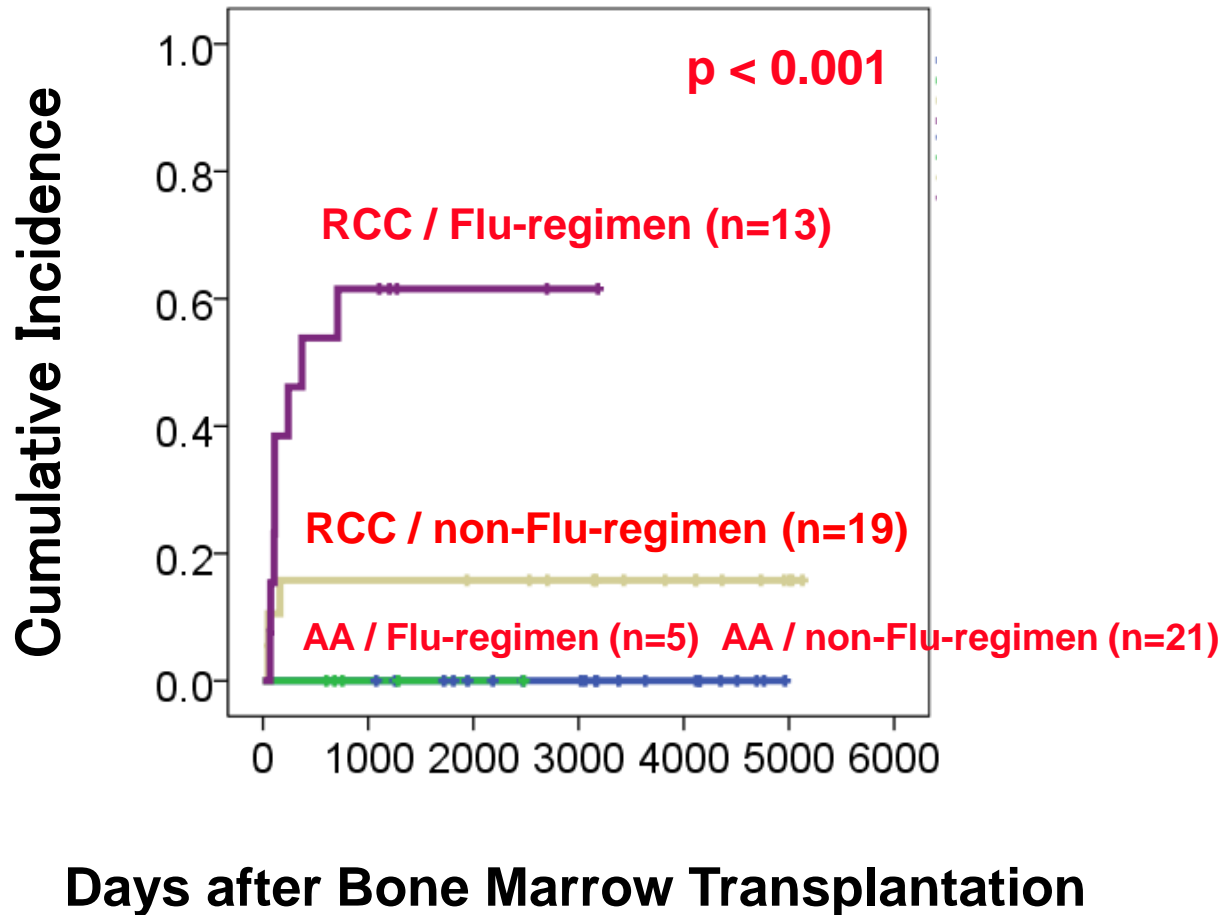
	<b>p</b>	<b>HR</b>	<b>95% CI</b>
<b>Flu regimen</b>	<b>0.023</b>	<b>4.68</b>	<b>1.23–17.8</b>
<b>RCC</b>	<b>0.931</b>		
<b>BMT after 2004</b>	<b>0.207</b>		
<b>TBI</b>	<b>0.334</b>		
<b>From Dx to BMT &lt;1000 days</b>	<b>0.481</b>		

# Cumulative Incidence of Donor-type Bone Marrow Failure



**Days after Bone Marrow Transplantation**

# Cumulative Incidence Donor type Bone Marrow Failure in the AA or RCC group



# Conditioning Regimens for UBMT in SAA patients

- #1 North America**  
**CY(50~100mg/kg) + Flu (120mg/m<sup>2</sup>) + TBI (2Gy) +ATG**
- #2 Europe**  
**CY (40mg/kg) + Flu (120mg/m<sup>2</sup>) + TBI (2Gy) +ATG**
- #3 Japan**  
**CY (100mg/kg) + Flu (100mg/m<sup>2</sup>) + TBI (3Gy) +ATG**

**Fludarabine**

**or**

**Decreased dose of cyclophosphamide**

# Patients and Methods

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- The clinical data of 660 patients with AA younger than 20 years who received BMT and registered in the Japan Society for Hematopoietic Cell Transplantation Registry from 1980 to 2010 was reviewed.
- The influence of potential risk factors on primary graft failure, donor-type bone marrow failure, OS and EFS was assessed according to clinical characteristics including conditioning regimen.

# • Patient characteristics

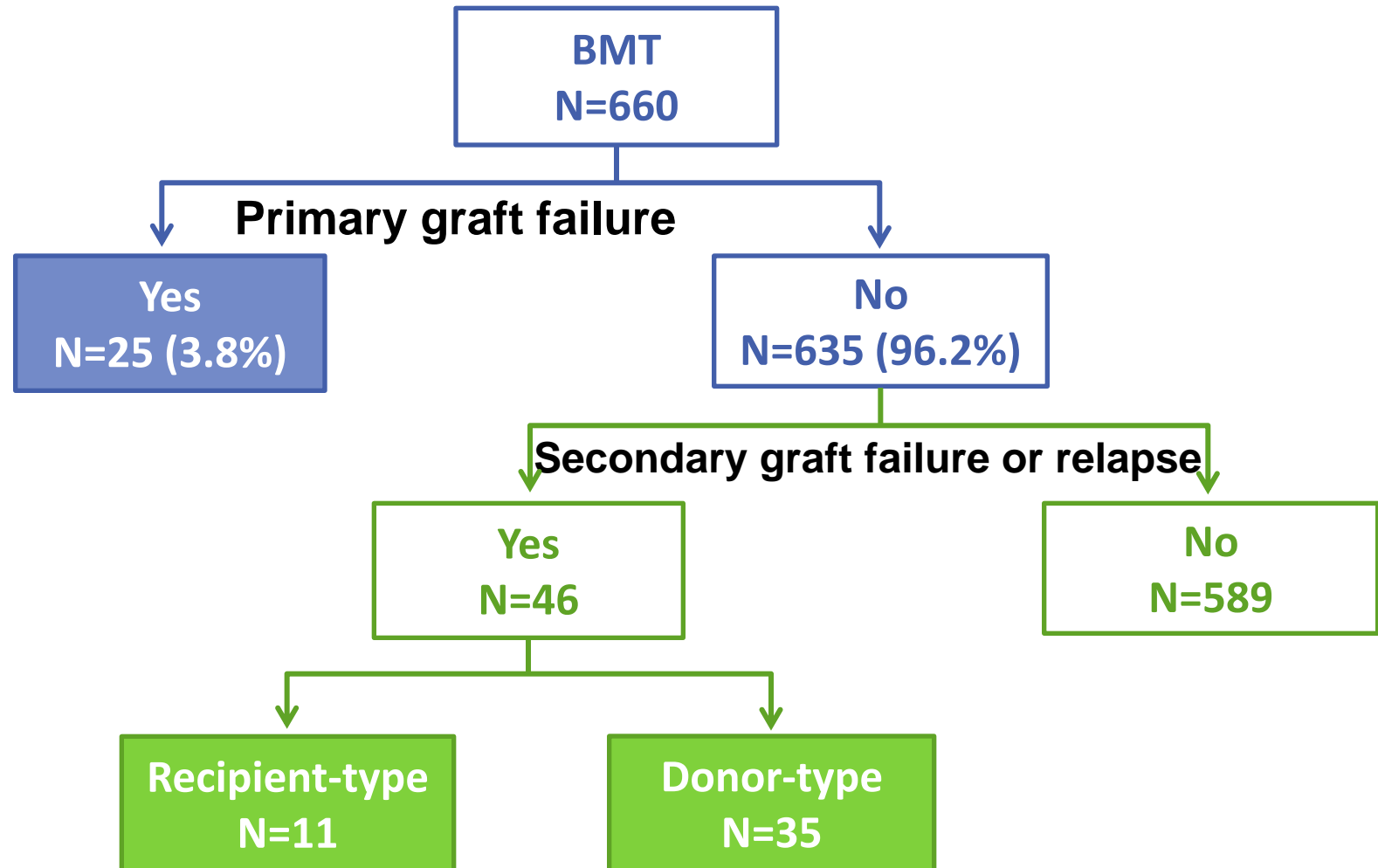
No. of patients	660
Age at BMT, years, median (range)	11 (0-19)
Gender male / female	377 / 283
Etiology, no. of patients (%)	
Idiopathic	615 (93.2)
Hepatitis	36 (5.5)
Others	9 (1.3)
Severity of AA at BMT, no. of patients (%)	
VSAA	97 (14.7)
SAA	308 (46.7)
NSAA	85 (12.9)
Unknown	170 (25.7)
IST before BMT, yes/ no	238 / 422
Interval from diagnosis to BMT, days, median (range)	287 (6-5563)

# • Transplantation characteristics

Donor	Related/ Unrelated	419 / 241
HLA	Match / Mismatch	497 / 163
ABO	Match / Mismatch / Unknown	343 / 281 / 36
Infused cell dose, x10 <sup>8</sup> /kg, median (range)		3.2 (0.6-13.0)
Conditioning		
	CY ± TLI	203
	CY + ATG ± TBI	230
	FLU + CY ± TLI	73
	FLU + CY + ATG ± TBI	137
	FLU + LPAM ± ATG ± TBI	9
	Others	8
GVHD prophylaxis		
	CyA alone	50
	CyA + MTX	384
	FK + MTX	211
	Others	15

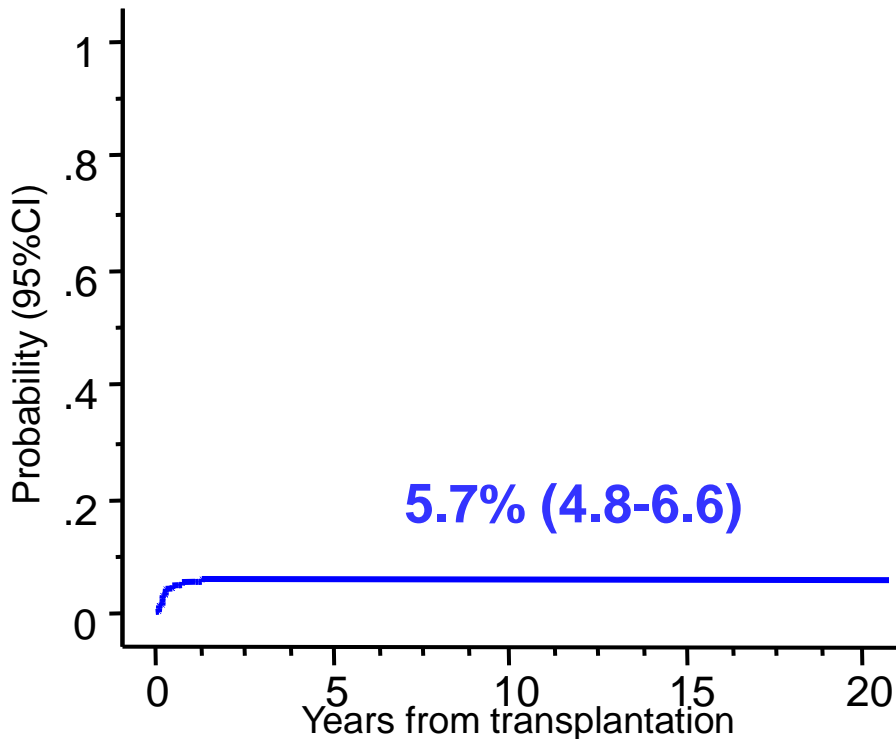


# Results

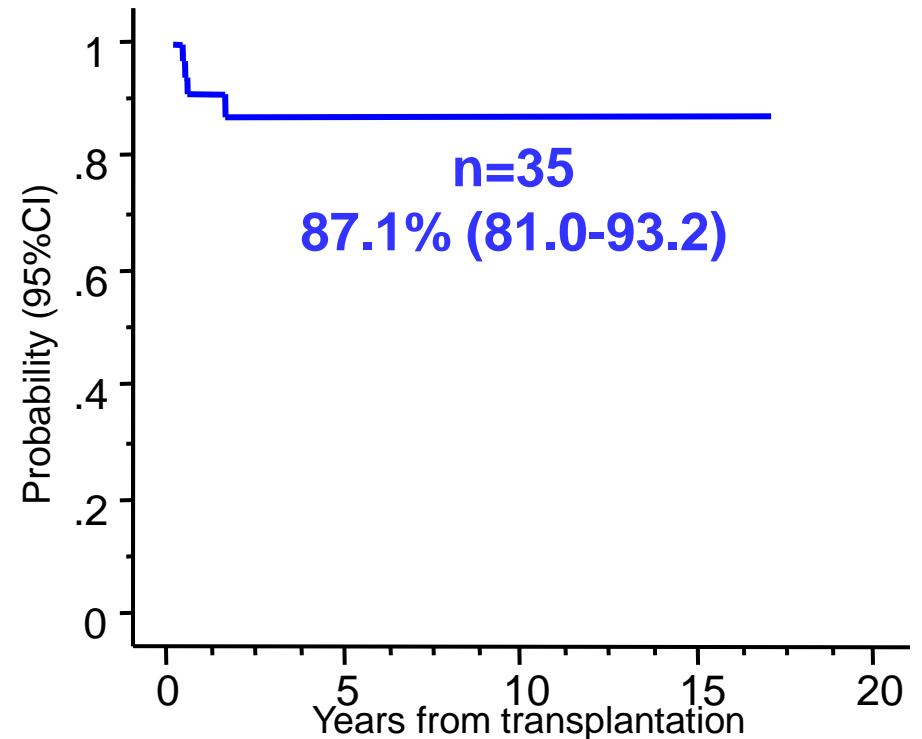


- Donor-type bone marrow failure

- Cumulative Incidence



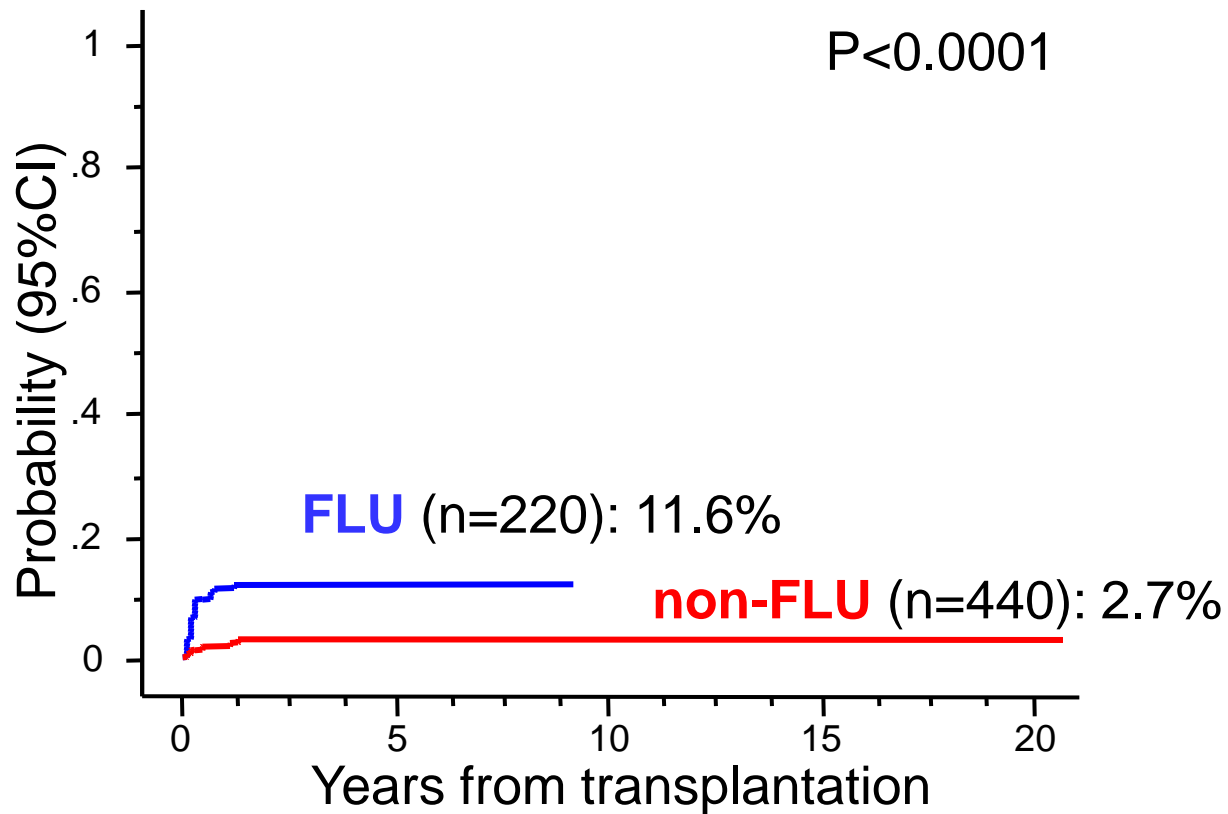
- Overall Survival



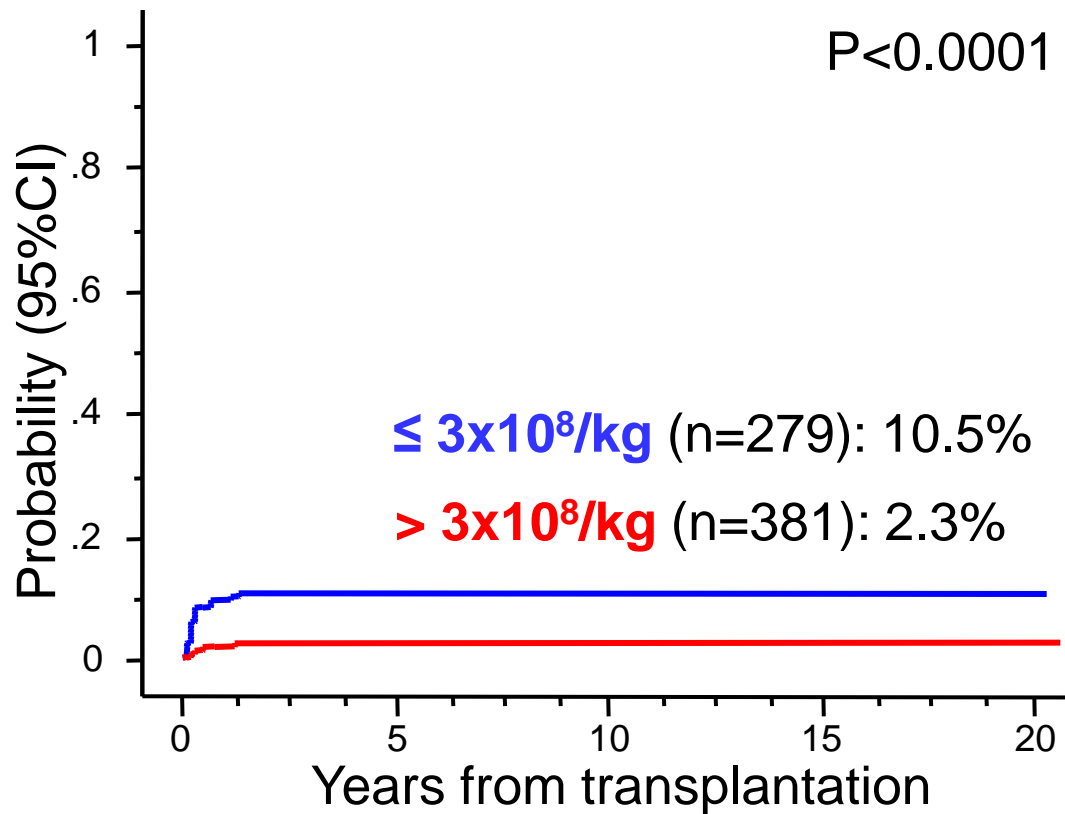
# • Variables

- Age at BMT
- Gender
- Etiology
- Severity at BMT
- Interval diagnosis-BMT
- RBC transfusion times
- Platelet transfusion times
- IST before BMT
- Donor: Related/Unrelated
- HLA: match/mismatch
- ABO: match/mismatch
- Infused cell dose
- Conditioning:
  - FLU
  - ATG
  - Irradiation
- GVHD prophylaxis
- Acute GVHD
- Chronic GVHD
- CMV antigenemia
- GCV

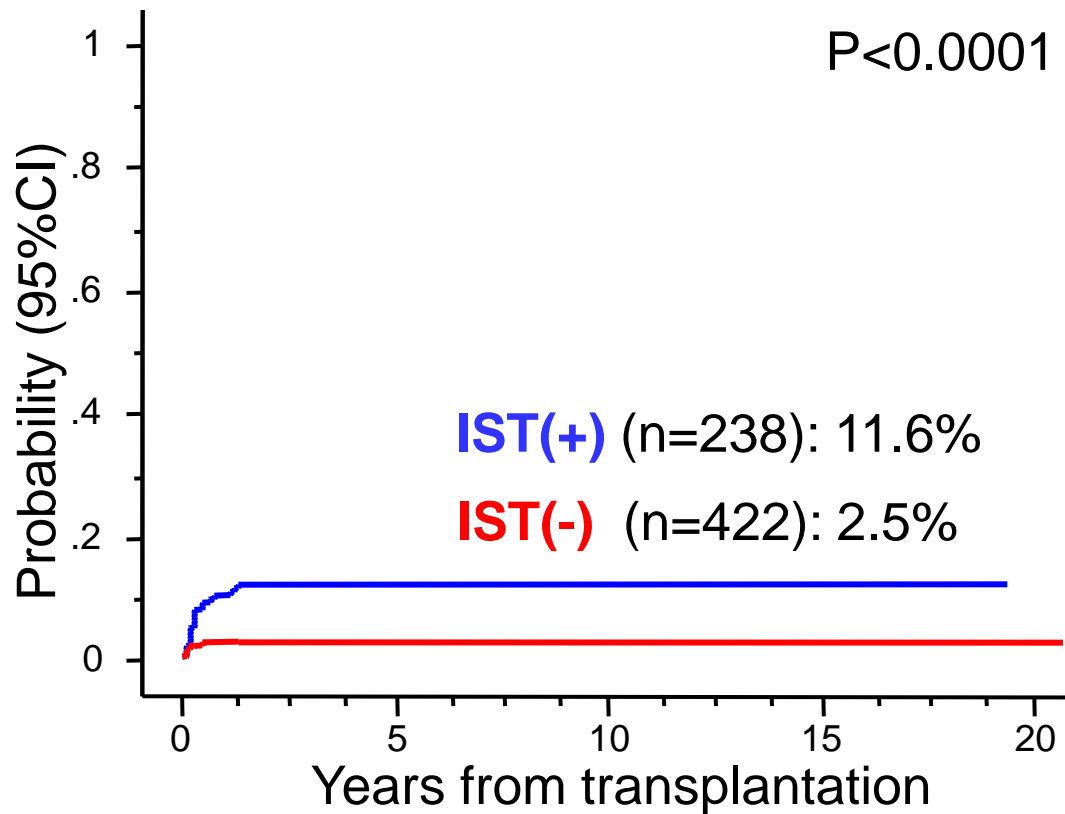
# • Cumulative Incidence: FLU vs. non-FLU



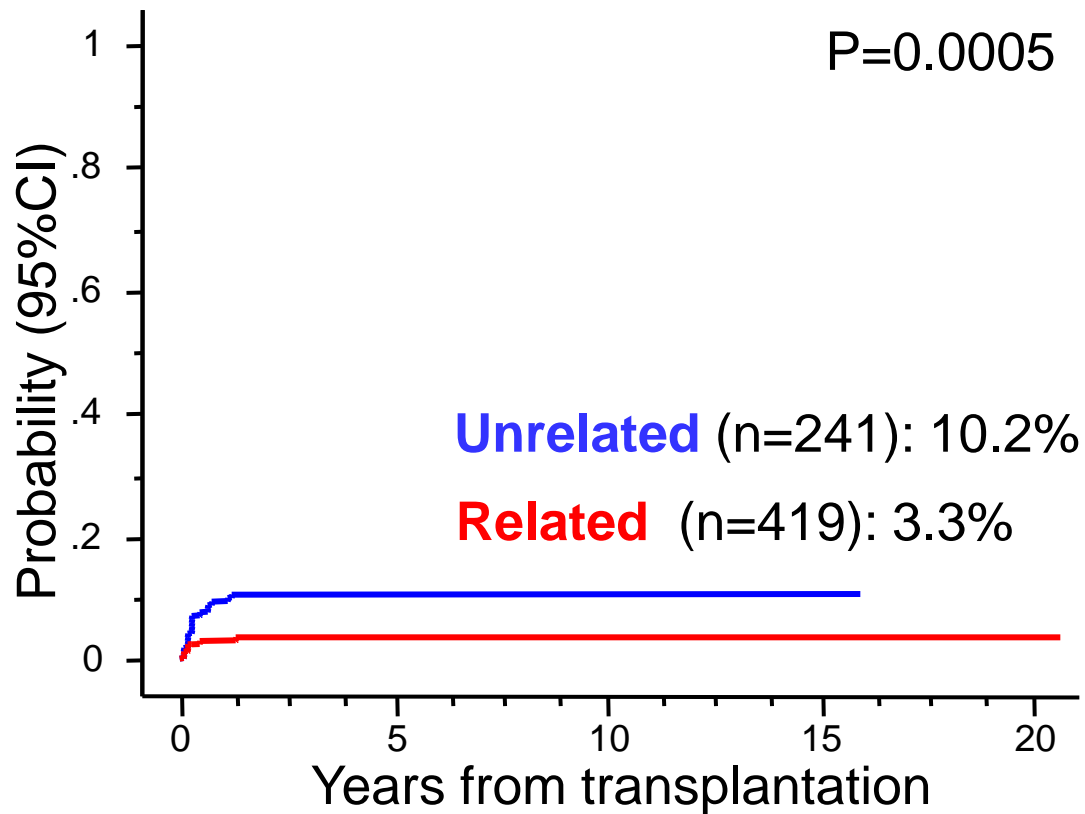
- Cumulative Incidence: Infused cell dose



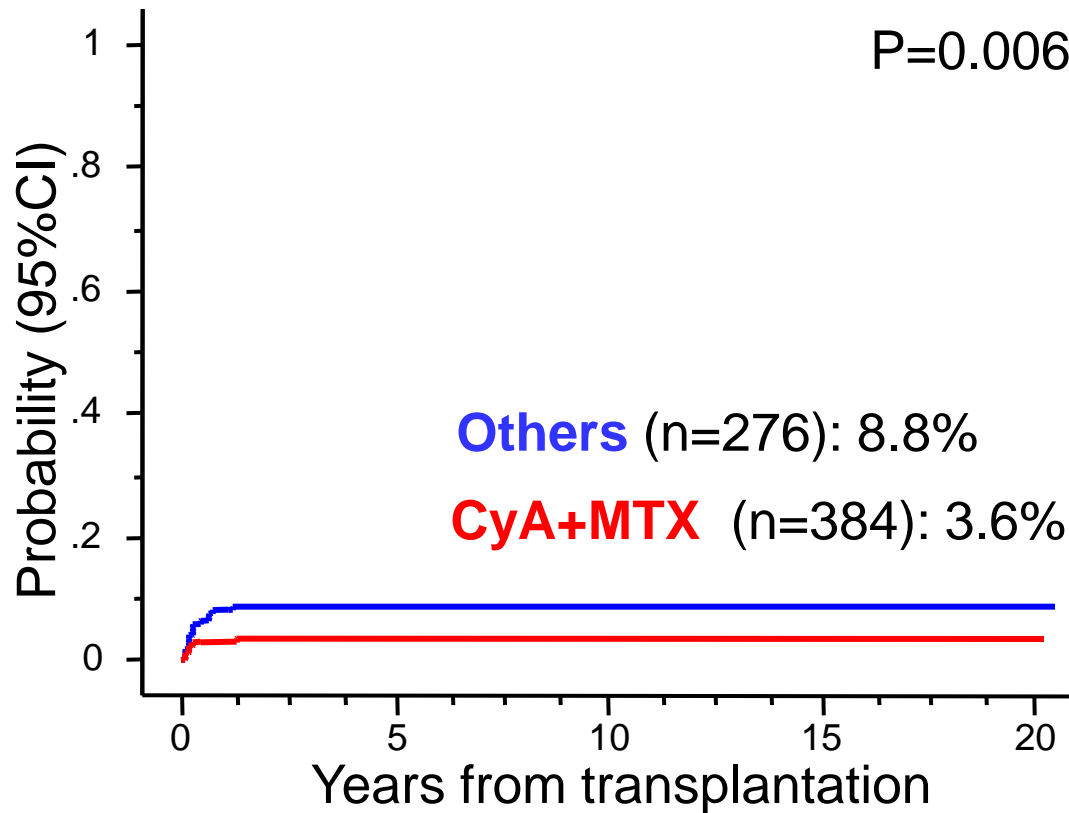
- **Cumulative Incidence: IST before BMT**



# • Cumulative Incidence: Donor

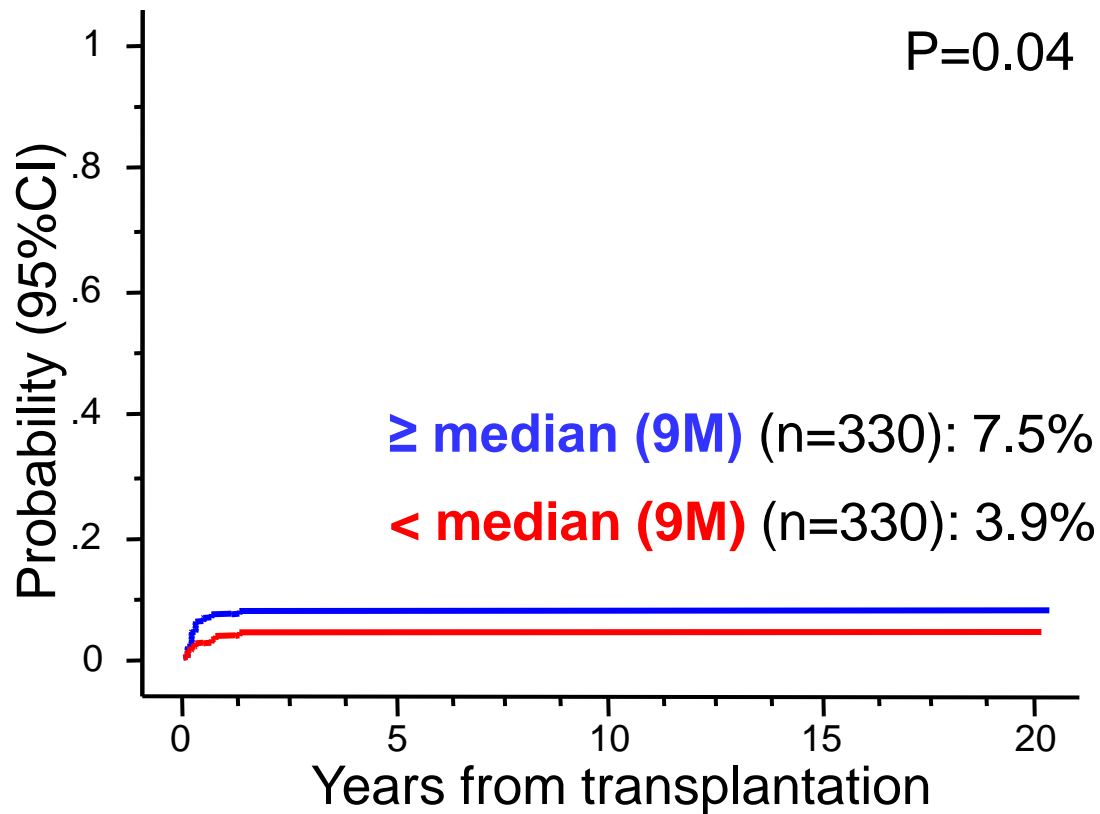


# • Cumulative Incidence: GVHD prophylaxis





# • Cumulative Incidence: Interval Dx-BMT



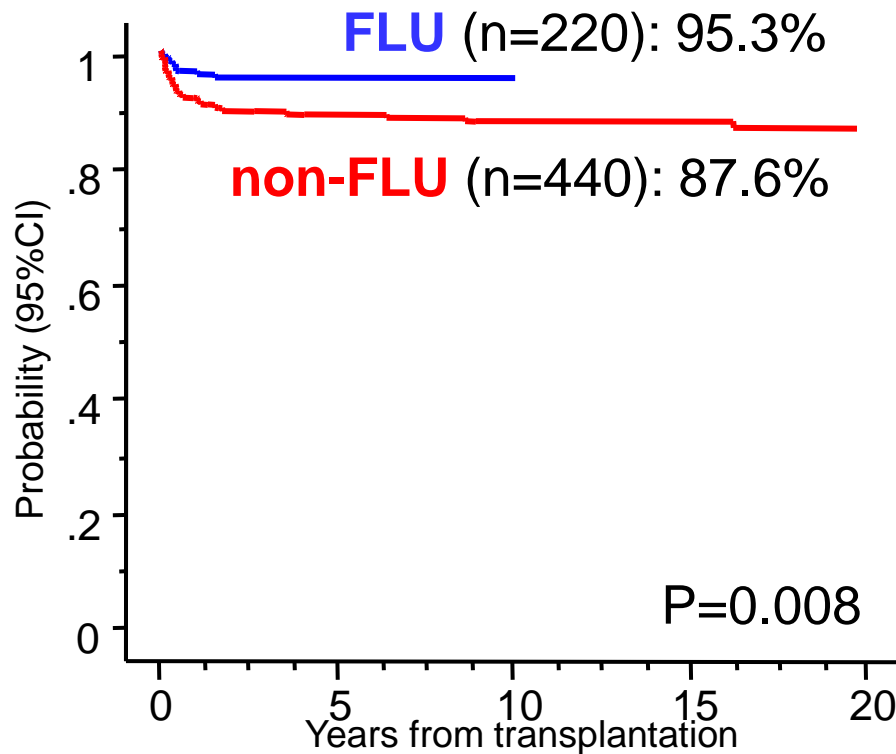
# • Multivariate analysis

## - Unfavorable factors for donor-type bone marrow failure

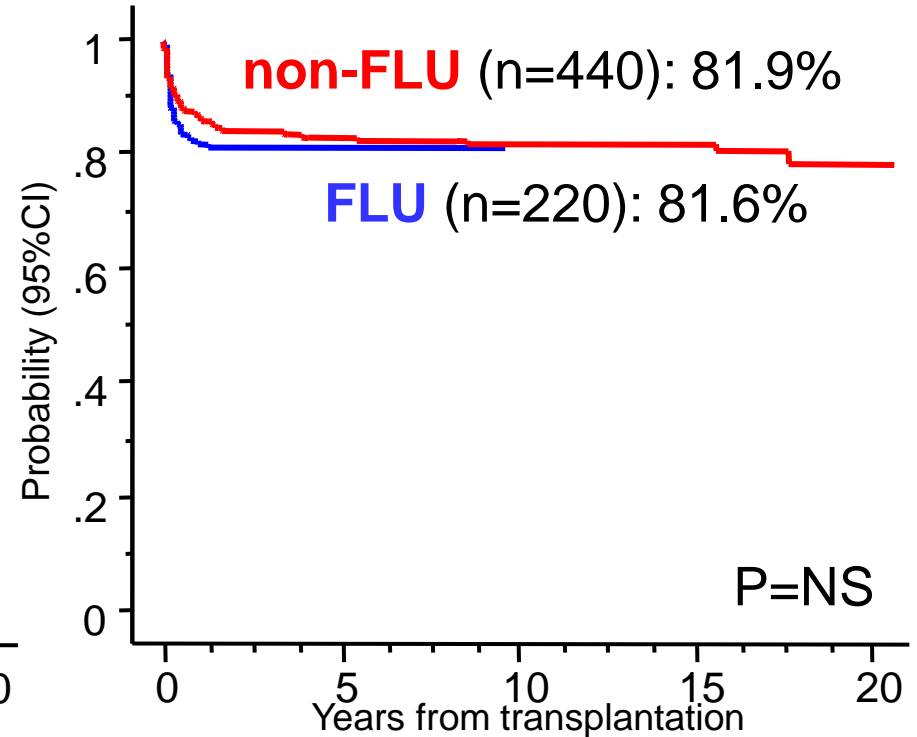
Variable	Odds ratio	95% CI	P
Infused cells, $\leq 3 \times 10^8/\text{kg}$	3.048	1.332-6.978	0.008
Conditioning, FLU regimen	2.351	1.044-5.296	0.039
IST before BMT, IST(+)	2.322	1.033-5.217	0.041
Donor, unrelated	1.670	0.669-4.169	NS
ABO, mismatch	1.590	0.795-3.178	NS
Age at transplantation, $\geq 10\text{y}$	1.341	0.623-2.883	NS
Conditioning, ATG regimen	1.231	0.577-2.626	NS
Interval diagnosis-BMT, $\geq$ median days	1.086	0.511-2.306	NS
GVHD prophylaxis, CyA+MTX	1.038	0.411-2.623	NS

- Impact of FLU regimen

- OS



- EFS



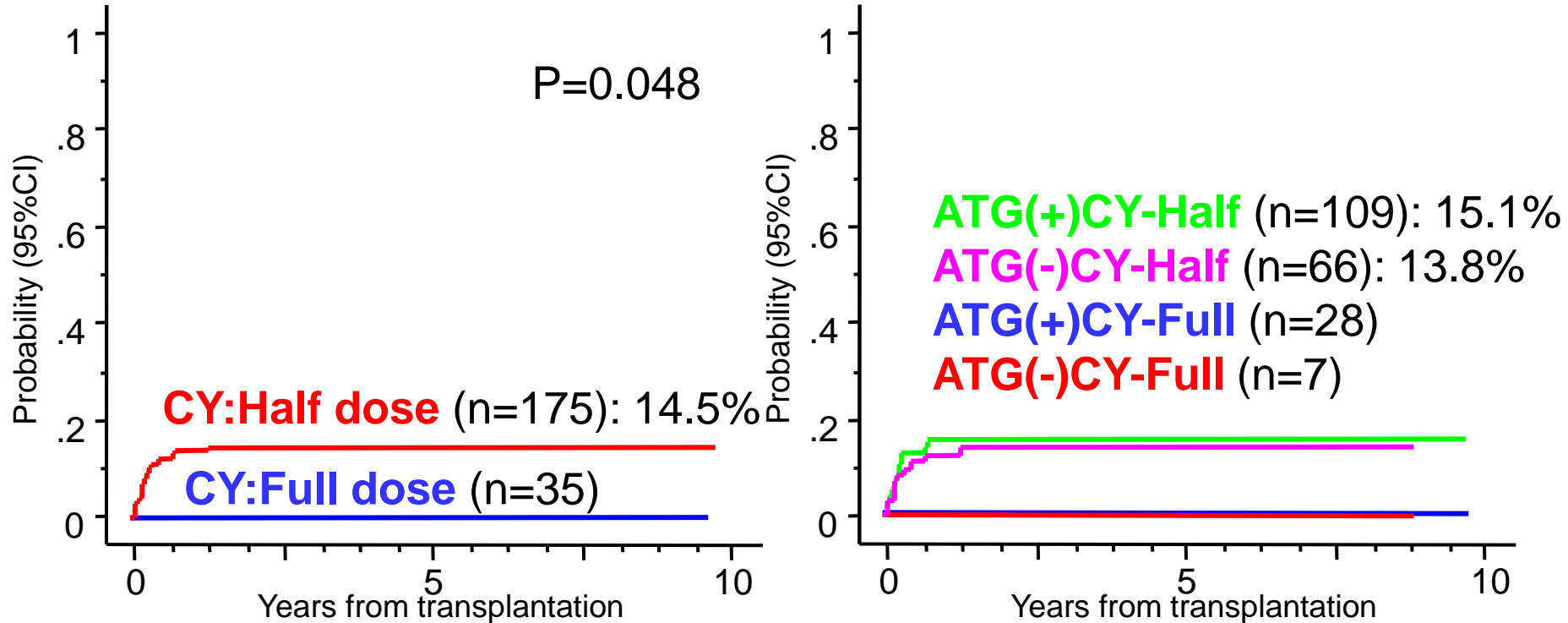
# • Impact of FLU regimen

## - Multivariate analysis of favorable factors

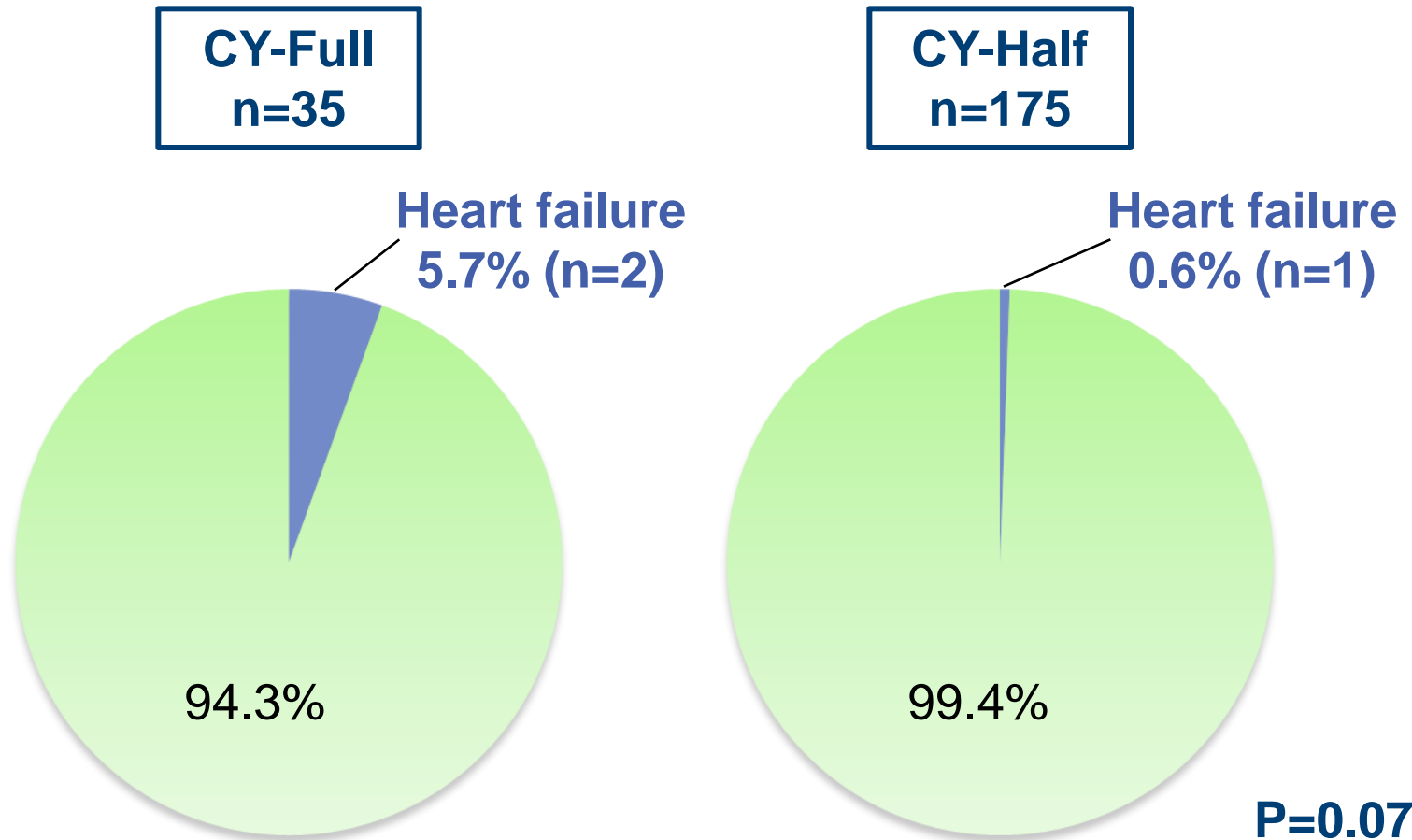
Primary engraftment	<i>P</i>
PC infusion, < 20 times	0.036
Conditioning, FLU regimen	0.089
OS	<i>P</i>
cGVHD, (-)	0.009
aGVHD, (-)	0.025
Conditioning, FLU regimen	0.046
PC infusion, < 20 times	0.081
EFS	<i>P</i>
Donor, related	0.002
Conditioning, FLU regimen	0.584

# • Impact of CY dose in FLU group

## - Cumulative Incidence of donor-type bone marrow failure

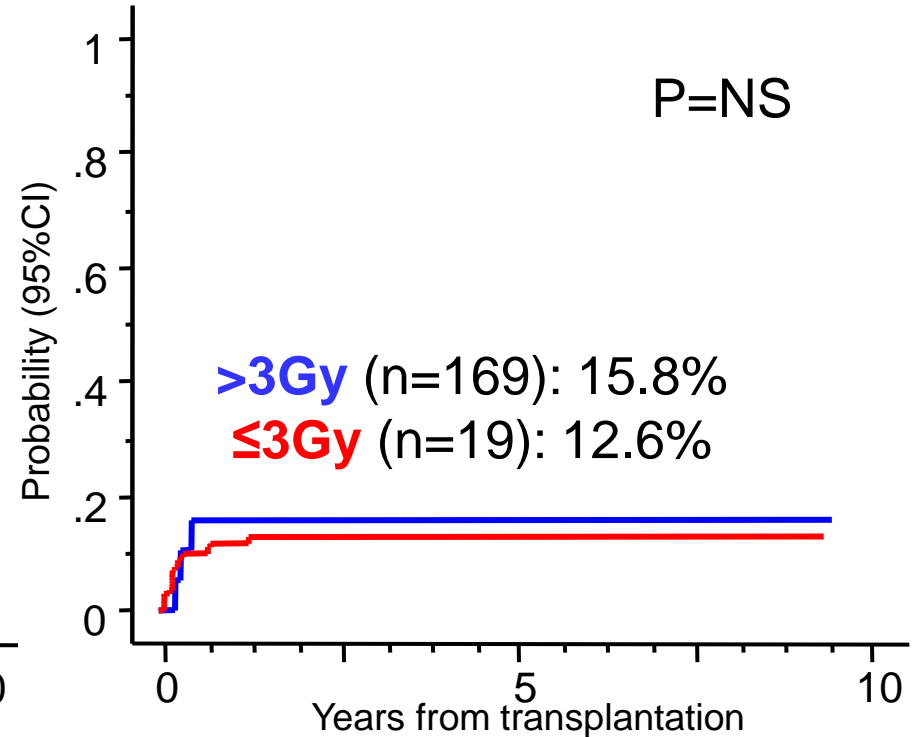
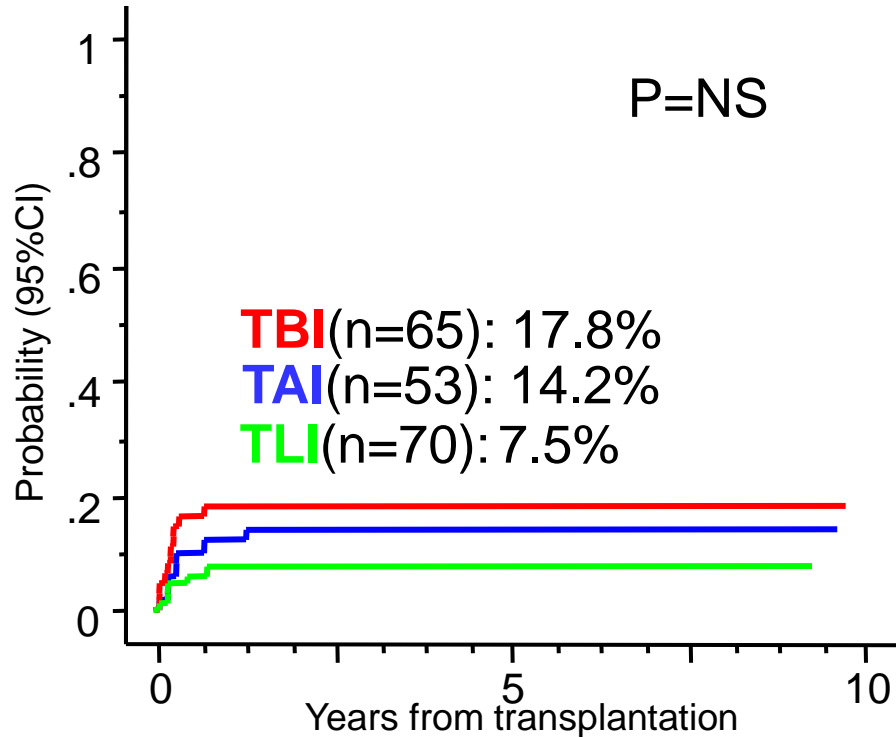


- Heart failure due to CY in FLU group



# • Impact of irradiation in FLU group

## - Cumulative Incidence of donor-type bone marrow failure



# Conclusion

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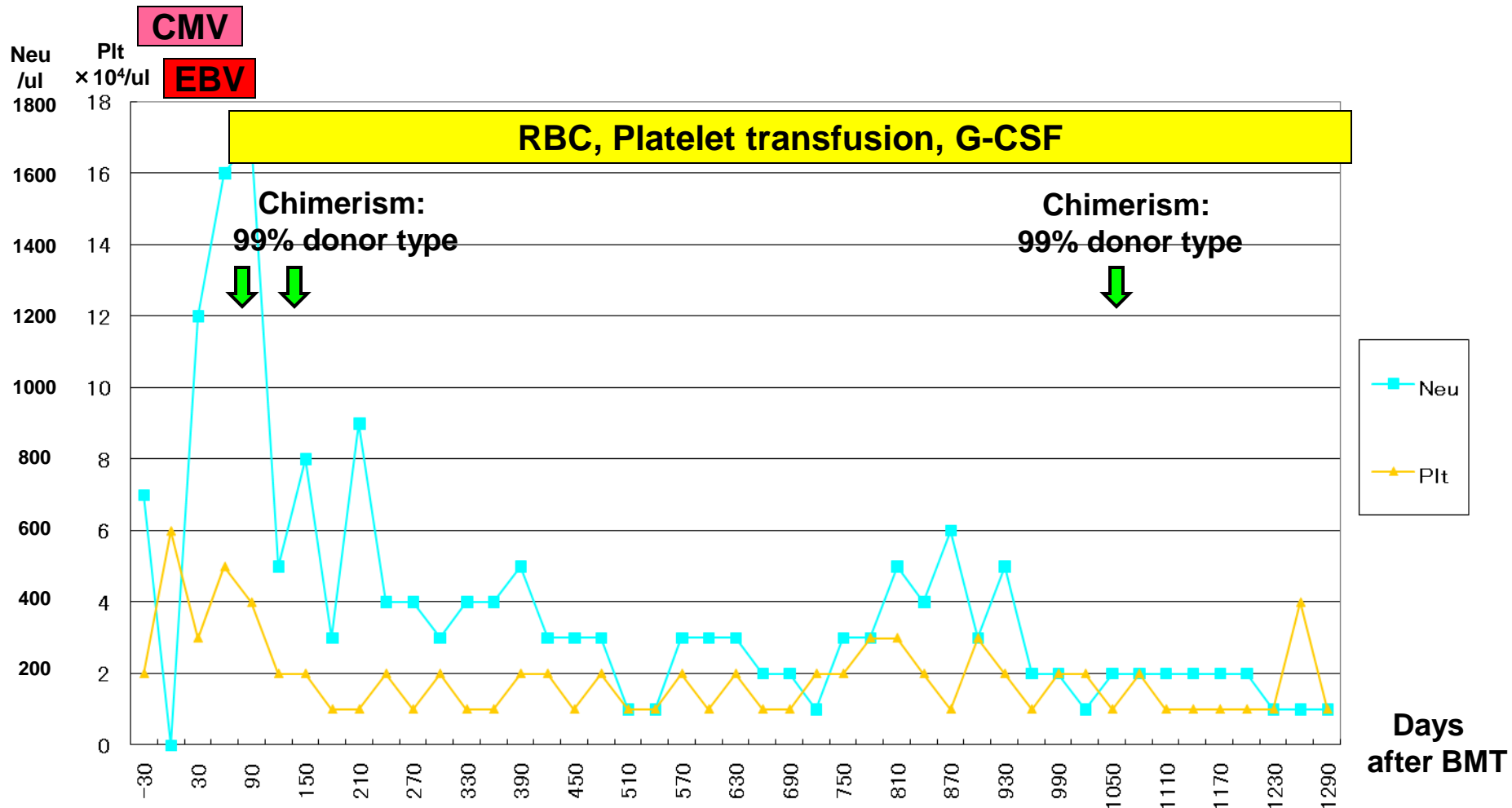
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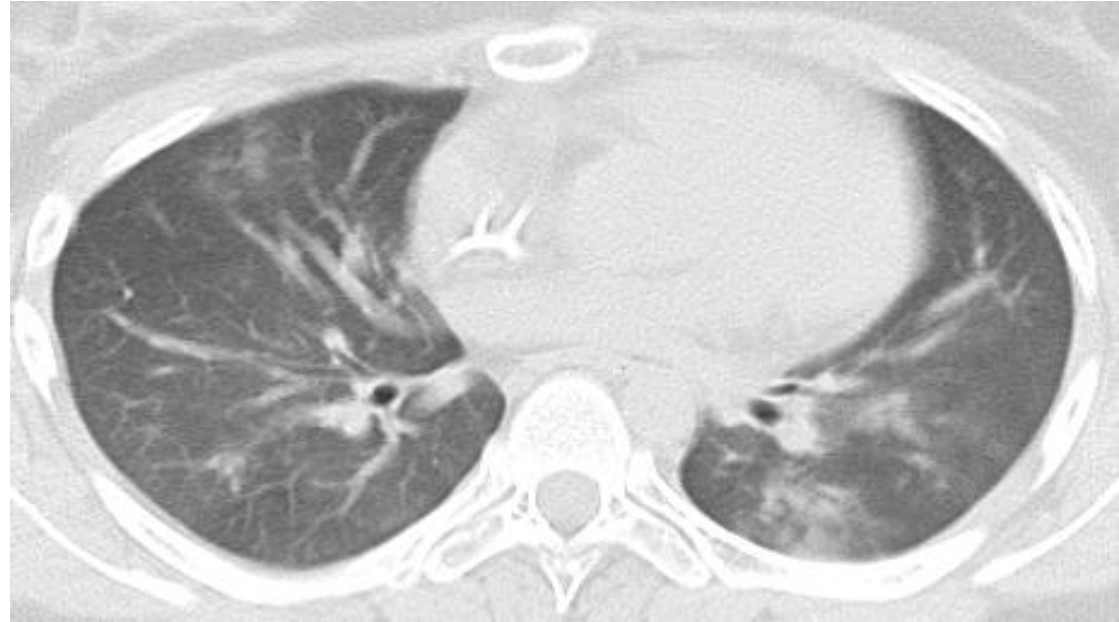
- FLU regimen was identified as a risk factor for donor-type bone marrow failure after BMT in children with AA, which could possibly be ascribed to the dose reduction of CY.
- However, the high incidence of heart failure after the regimen included full dose of CY in combination with FLU is an important issue to be resolved.
- To develop optimal treatment, we need to reconsider the conditioning regimen for children with AA.



# Clinical course

11yF, RCC, Donor: matched unrelated,  
Conditioning regimen: FLU+CY+Campath+TBI(3Gy),  
GVHD prophylaxis: FK506, Cell dose:  $1.5 \times 10^8/\text{kg}$ , aGVHD: grade 1,  
CMV antigenemia (+), EBV-LPD (+), Onset of aplasia after BMT: day110



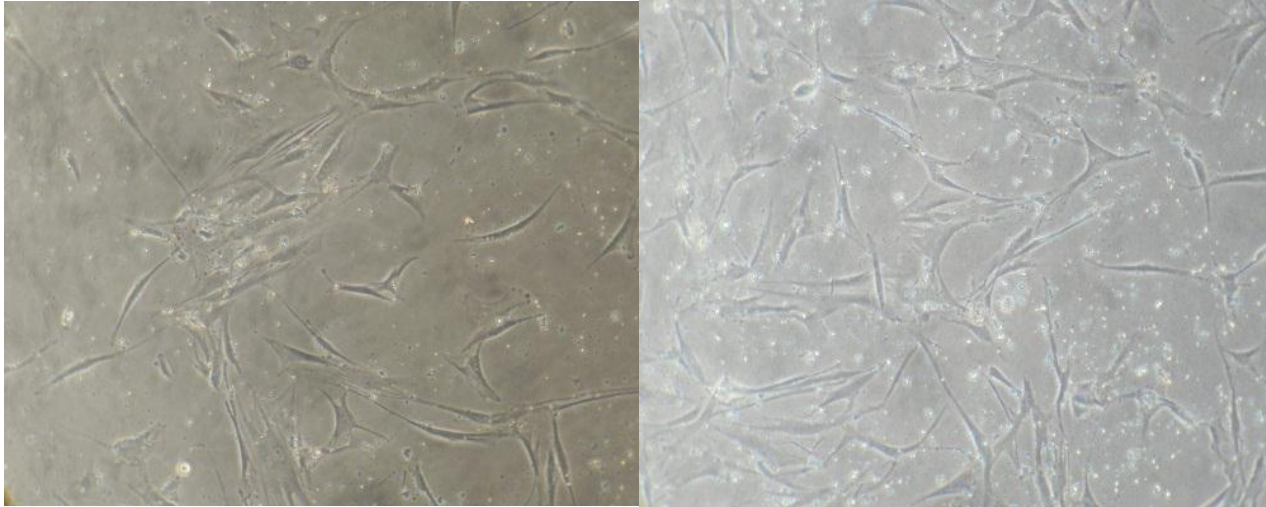


Day 1360

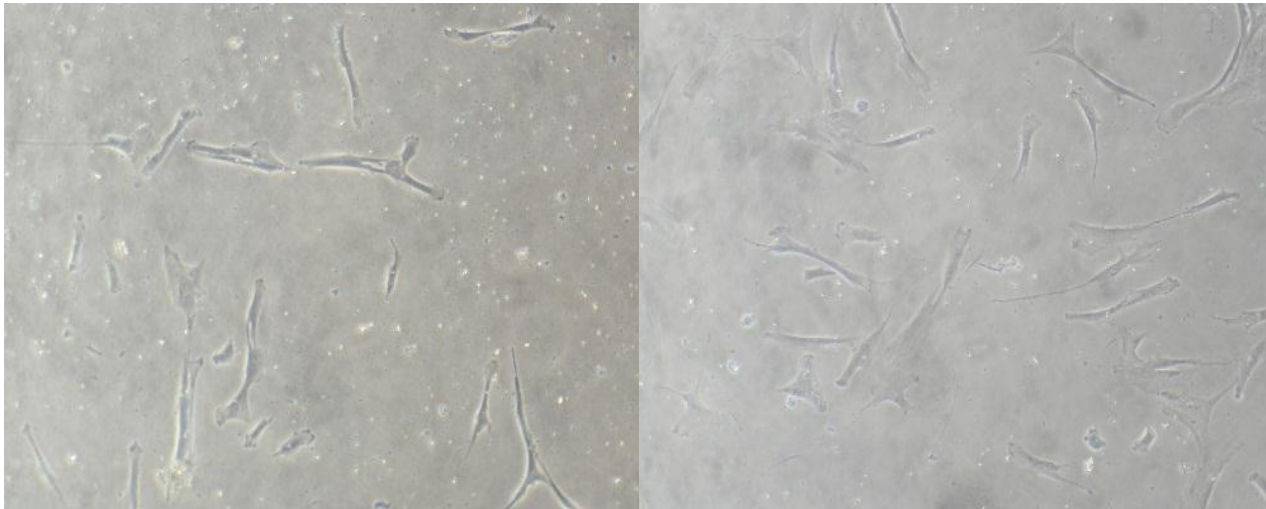
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# Bone Marrow Mesenchymal Stem Cell in SAA Patients with Donor Type Bone Marrow Failure

Normal 1



Patient 2

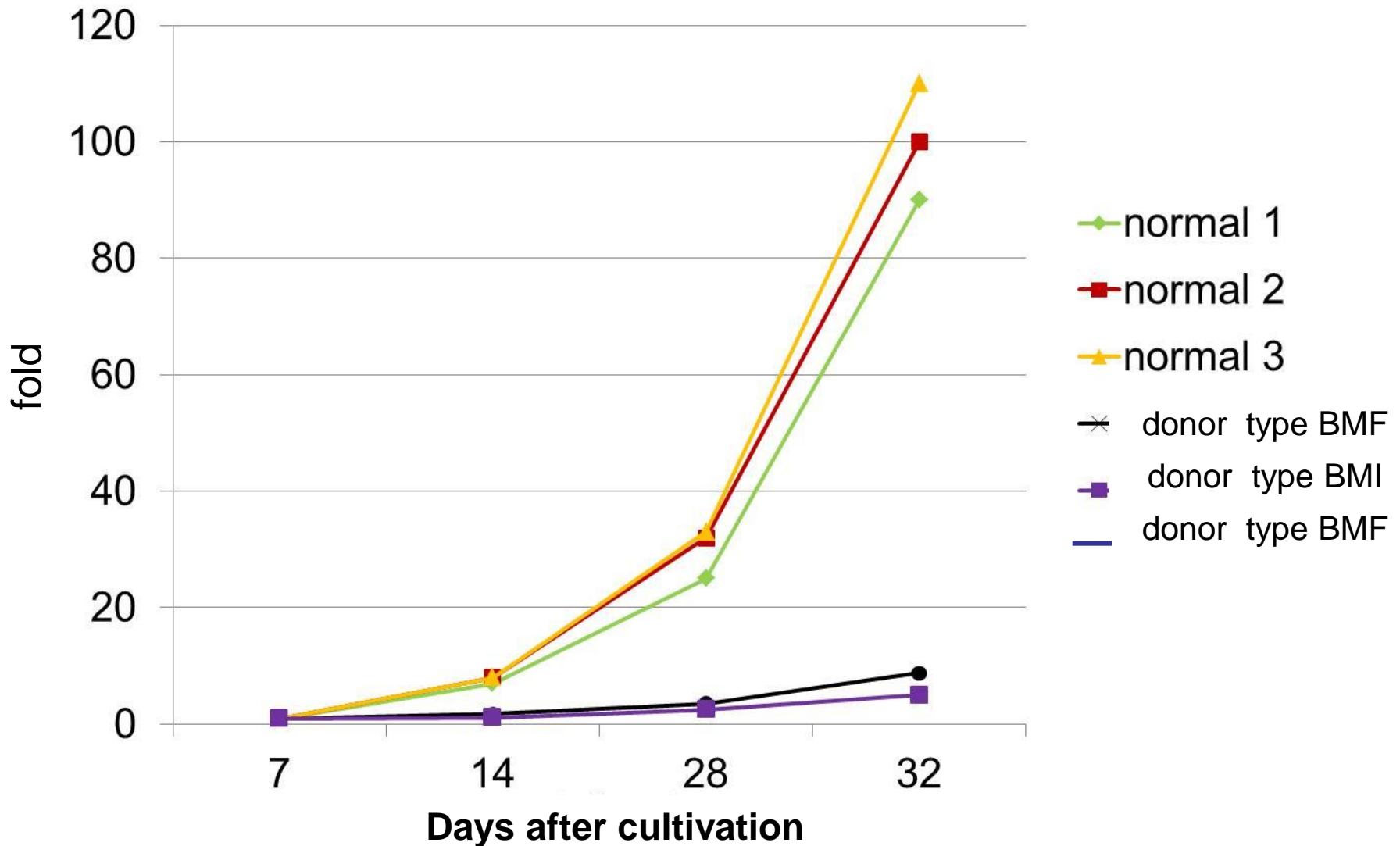


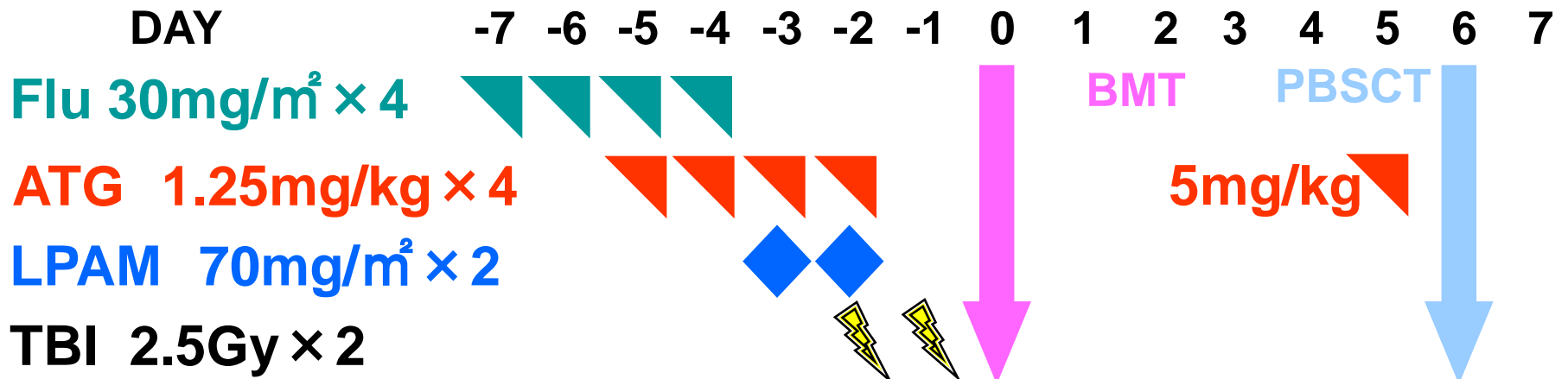
Day 3

Day 7



# Bone Marrow Mesenchymal Stem Cell in SAA Patients with Donor Type Bone Marrow Failure





## BMT Setting

**Donor : Father**

	A	B	Cw	DR
<b>Resipient :</b>	<b>2402/3101</b>	<b>5101/5601</b>	<b>0401/1402</b>	<b>0405/0901</b>
<b>Donor :</b>	<b>3101/3101</b>	<b>5101/5601</b>	<b>0401/1402</b>	<b>0401/0901</b>

**gender : F to M**

**blood : minor(donorO+)**

**GVHD prophylaxis**

- short MTX day1, 3, 7, 11
- FK506

## MSC

NCC  $0.75 \times 10^6/\text{kg}$

Viability 76.1%

## PBSCT

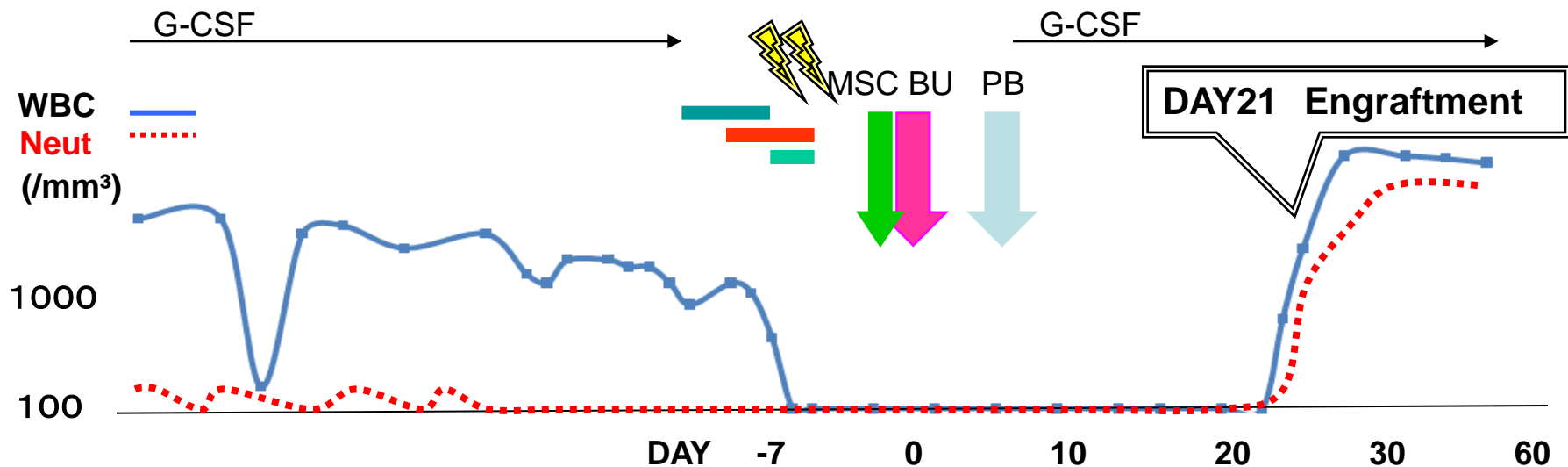
NCC  $3.0 \times 10^8/\text{kg}$

CD34  $2.24 \times 10^6/\text{kg}$

## BMT

NCC  $2.84 \times 10^8/\text{kg}$

CD34  $1.10 \times 10^6/\text{kg}$



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- JSHCT pediatric aplastic anemia WG

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