upregulation of KLF2 at mRNA (Figure 1a) and protein (Figure 1b) levels in all the five described cancer cell lines. Upon EZH2 knockdown at 72 h, we also observed a defect on cell viability determined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and an increase in G1/decrease in S-phase assessed by fluorescence-activated cell sorting analysis (Supplementary Figure 1). We strengthened the link between EZH2 expression and KLF2 repression by establishing a U2OS cell line stably transfected with a short hairpin RNA (shRNA) against EZH2 (Figure 1c and Supplementary Figure 2). EZH2–shRNA (Origene, Rockville, MD, USA) transfection was accomplished by electroporation and cells were selected with puromycin (Calbiochem, Darmstadt, Germany). The stable inhibition of EZH2 expression also led to a marked increase in KLF2 mRNA (Supplementary Figure 2) and protein levels (Figure 1c).

The observed inverse association between EZH2 and KLF2 levels might be mediated by a direct effect of EZH2 on the KLF2 promoter or by secondary mechanisms. Thus, we performed quantitative chromatin immunoprecipitation (qChIP) for the minimal promoter of KLF2 using antibodies against EZH2, the trimethylated H3K27 mark (3meH3K27) established by the enzyme and phosphorylated serine 2 of RNA polymerase (RNAP-S2, a marker of active transcription). A nonspecific IgG antibody was used as a technical negative control, the EZH2-target gene *ADRB2* (Yu *et al.*, 2007) was used as a positive control and the GAPDH locus was used as a negative control. Measurements were made in triplicate and

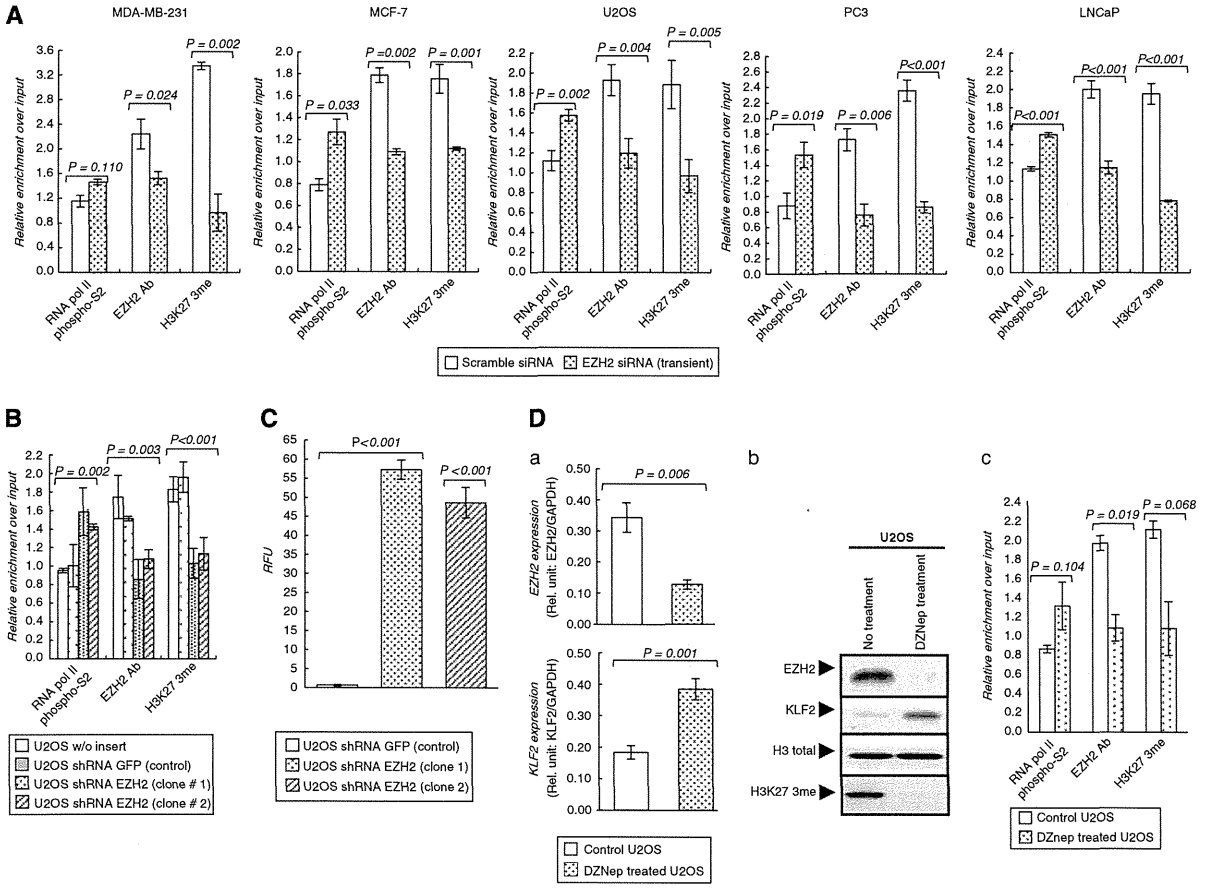

Figure 2 EZH2 direct binding to the KLF2 promoter mediates the transcriptional repression effect. (**A**) ChIP–qPCR of EZH2 occupancy and H3K27-3me marks in the *KLF2* promoter in five cancer cell lines treated with EZH2 siRNA (72 h) or scrambled siRNA. Treatment with siRNA against EZH2 prevents EZH2 occupancy and the presence of the H3K27-3me mark, while enhanced RNAP binding is observed in the *KLF2* promoter. ChIP was performed using polyclonal antibodies raised in rabbit against EZH2 (pAb-039-050, Diagenode, Liège, Belgium), RNAP-S2 (ab5095, Abcam, Cambridge, UK) and H3K27me3 (pAB-069-05, Diagenode), with rabbit IgG as a control (ab37415, Abcam, ChIP grade). The primers used for the ChIP–qPCR analysis of the KLF2 promoter were 5′-GAGACTCCAGACT TCCCATCC-3′ (sense) and 5′-CAGAGACTCTCAGGGGAGCAC-3′ (antisense). (**B**) qChIP for EZH2 occupancy and H3K27-3me presence for the *KLF2* promoter in stable EZH2 knockdown clones (U2OS-shEZH2). (**C**) *KLF2* promoter activities are analyzed by luciferase reporter assay in stable EZH2 knockdown clones. In each experiment, firefly luciferase activities are normalized against those of Renilla. $n = 3$, mean ± s.e.m. (error bars). We used a pGL3 Luciferase Reporter Vector (Promega, Madison, WI, USA) for the KLF2 promoter encompassing *Nhe*I/*Hin*d III sites (from −916 to +129 bp). (**D**) Upregulation of *KLF2* transcript (a) and protein (b) upon treatment with 5 μM DZNep for 72 h. (c) qChIP analysis shows how the treatment with DZNep decreased EZH2 occupancy and the H3K27-3me mark in the KLF2 promoter, while it enhanced RNAP-S2 occupancy. *P*-values obtained from Student's *t*-test.

the polymerase chain reactions (PCRs) were done using the Prism 7900 HT Sequence Detection System (Applied Biosystems, Carlsbad, CA, USA). The qChIP analyses demonstrated an enriched presence of EZH2 and 3meH3K27 in the KLF2 promoter for the described cancer cell lines (Figure 2A). Conversely, RNAP-S2 was depleted at this locus (Figure 2A). The EZH2 RNAi experiments reduced EZH2 occupancy and 3meH3K27 presence and induced the recruitment of RNAP-S2 for the KLF2 promoter (Figure 2A). These results for the 5′-end of the *KLF2* gene were similar to those obtained from the qChIP data of the well-known EZH2-target gene *ADRB2* (Yu *et al.*, 2007) (Supplementary Figure 3). The U2OS cells that were stably depleted at EZH2 by shRNA reproduced this qChIP pattern (Figure 2B). In these cells, evidence for the role of EZH2 in directly repressing KLF2 was reinforced by the results of luciferase assays (Figure 2C). Finally, the link between EZH2 binding to the 5′-end of the KLF2 and its corresponding silencing was corroborated by the use of the small molecule 3-Deazaneplanocin A (DZNep), which depletes the cellular levels of Polycomb-repressive complex 2 components, including EZH2 (Tan *et al.*, 2007). Upon DZNep treatment, a marked increase in KLF2 expression was observed in U2OS cells (Figure 2D). The DZNep-mediated enhancement of KLF2 expression was associated with the depletion of EZH2 occupancy and 3meH3K27 levels and an

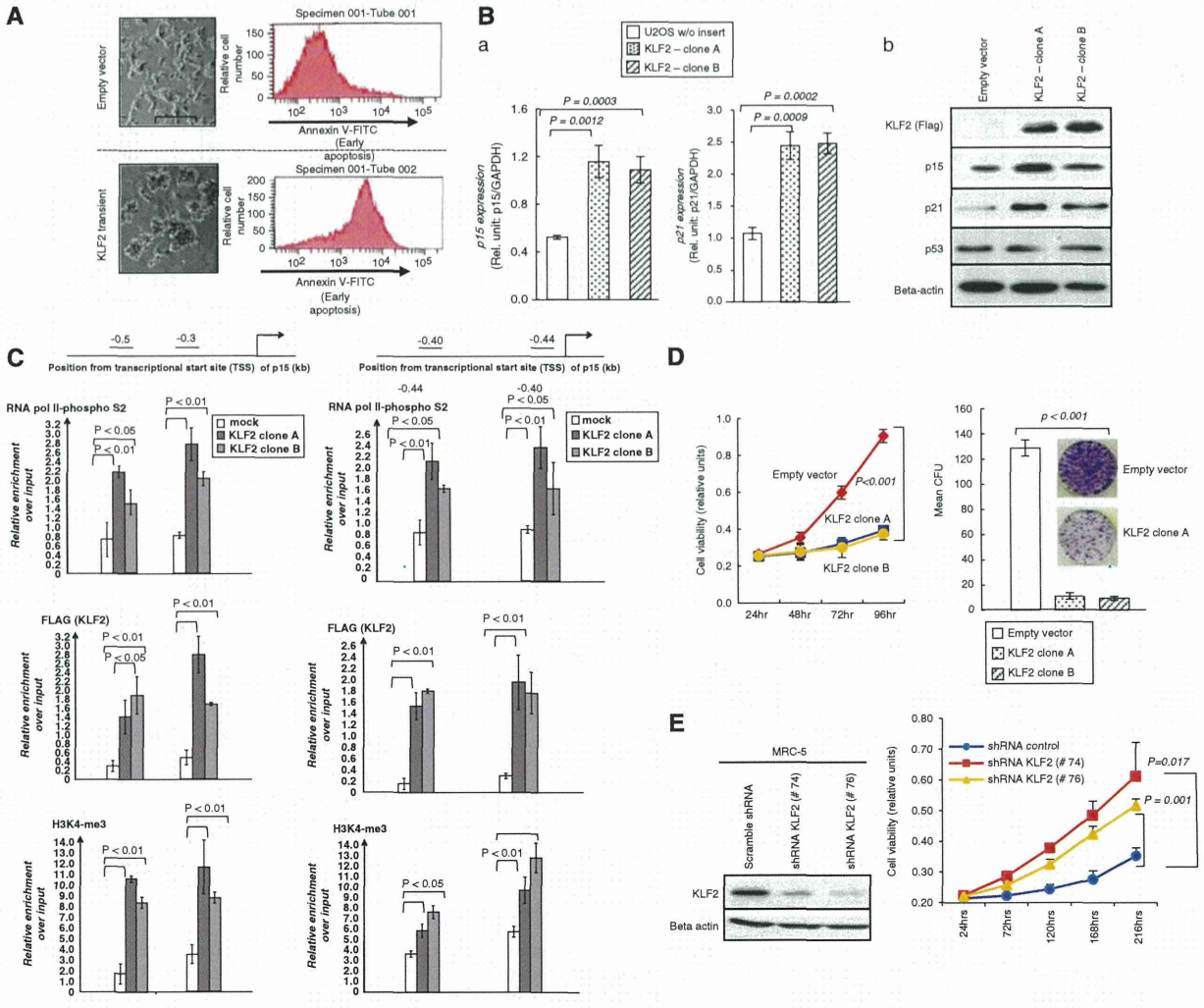

Figure 3 KLF2 induces apoptosis, directly activates the cell cycle-inhibiting genes p15/CDKN2B and p21/CDKN1A and inhibits cell proliferation. (**A**) Enhanced Annexin V expression relative to empty vector-transfected cells upon KLF2 transfection. The right panels are the original red color/FL-4 (annexin-Cy5) histograms that show how KFL2 transfection induces apoptosis, demonstrated by the increase in the number of cells that incorporate higher amounts of annexin V. (**B**) U2OS cells transfected with a FLAG-tagged KLF2 (pCMV-Tag2B-KLF2) expression vector show increased expression of $p15^{Ink4b}$ and $p21^{CDKN1A}$, determined by qRT–PCR (a) and western blot (b). (**C**) The ectopically expressed KLF2 (FLAG-tagged KLF2) occupied the $p15^{Ink4b}$ and $p21^{CDKN1A}$ promoters in association with a gain of RNAP-S2 occupancy and the H3-K4 trimethylation mark determined by qChIP. (**D**) MTT (left) and colony formation (right) assays reveal that stable KLF2-expressing U2OS cells grow more slowly than cells transfected with control vector. (**E**) Enhanced proliferation of normal MRC5 fibroblasts after transfection of shRNA against KLF2 in comparison with scrambled shRNA. *P*-values obtained from Student's *t*-test.

enrichment of RNAP-S2 in the KLF2 promoter determined by qChIP (Figure 2D). From the DNA methylation standpoint, the KLF 5′-end region contains a canonical CpG island that remained unmethylated under all the experimental conditions described (Supplementary Figure 4).

Once we had determined that KLF2 was a direct target of transcriptional repression by EZH2 in cancer cells, we sought to understand the molecular and cellular contribution of KLF2 silencing to the transformed phenotype. To this end we constructed a FLAG-tagged KLF2 expression vector using the pCMV-Tag2B vector (Stratagene, Santa Clara, CA, USA) and transfected it by electroporation to U2OS cells. Transfected cells were selected by adding G418 (Calbiochem). Upon KLF2 transfection, we observed enhanced Annexin V expression relative to empty vector-transfected cells (Figure 3A). The pro-apoptotic effect mediated by KLF2 transfection was also associated with an increase in the expression levels of the cell cycle-inhibiting genes p15/CDKN2B and p21/CDKN1A, as determined by qRT–PCR (Figure 3B) and western blot (Figure 3B). These two latter genes could be direct or indirect targets of the transcription factor KLF2. The two possibilities can be discriminated by the chromatin immunoprecipitation assay. The qChIP analyses demonstrated an enriched presence of KLF2 (using a FLAG-M2 antibody) in the 5′-end CpG islands of p15/CDKN2B and p21/CDKN1A in the transfected U2OS cells (Figure 3C). The KLF2 occupancy at these promoters

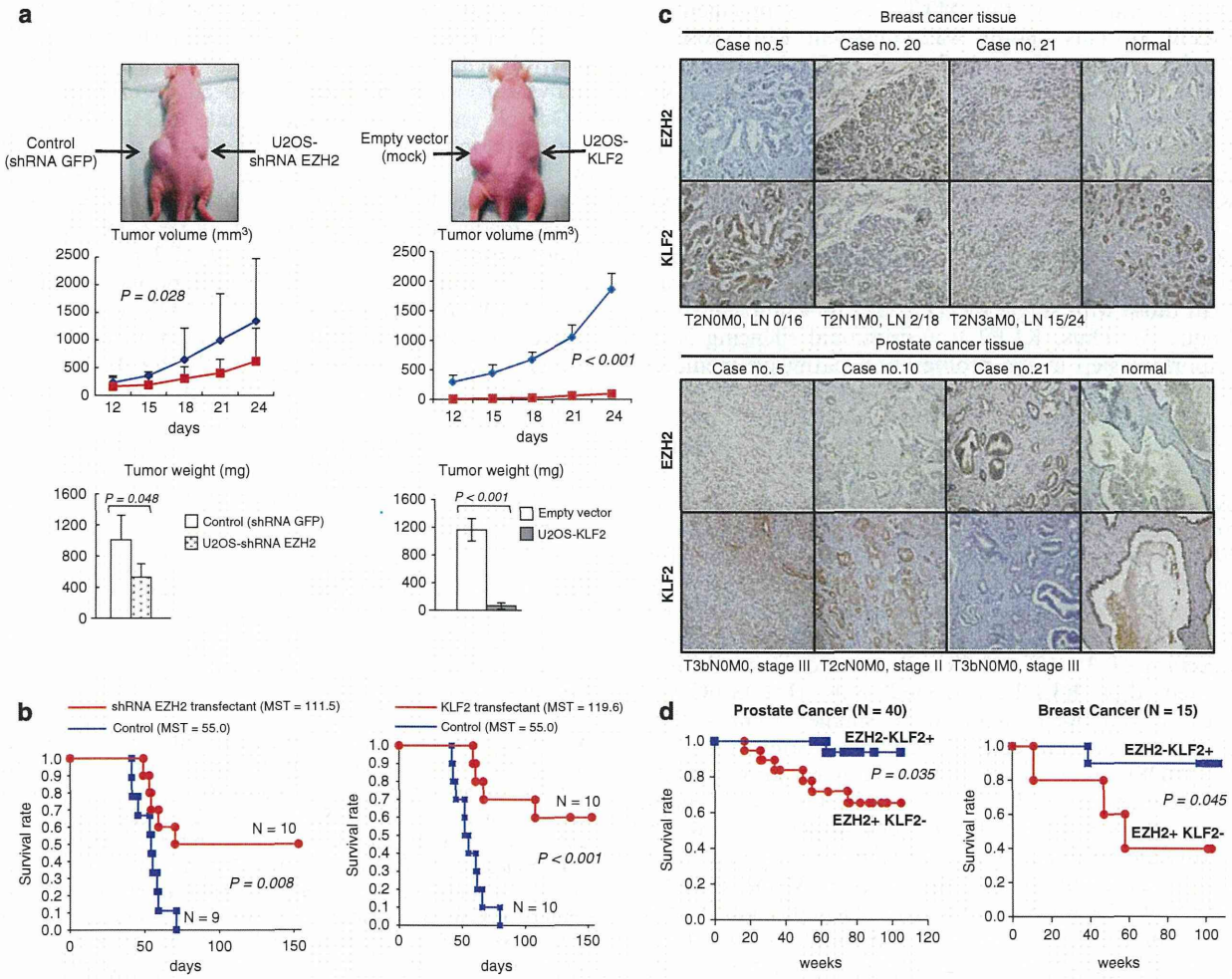

Figure 4 KLF2 as a tumor suppressor in mouse models and its effect on human tumors. (**a**) Effect of sh-EZH2 knockdown (left) or KLF2 transfection (right) on the growth of U2OS cells inoculated into nude mice. Tumor volume was monitored over time, and the tumor was excised and weighed after 24 days. EZH2 depletion or KLF2 overexpression cause a reduction in tumor volume and weight. (**b**) Significantly lower mortality following tail-vein injection in the mice of 1×10^6 U2OS cells was observed in U2OS-pCMV-KLF2 or U2OS-shEZH2 cells in comparison with the empty vector-transfected cells ($P<0.001$) (**c**) EZH2 and KLF2 expression in clinical cancer samples determined by immunostaining in prostate and breast cancer tissue microarrays. KLF2 expression was inversely associated with EZH2 expression in prostate (Pearson's correlation coefficient $r^2=0.32$, $P<0.05$, $n=40$) and breast (Pearson's correlation coefficient $r^2=0.57$, $P<0.05$, $n=15$) cancer. (**d**) The high expression of EZH2 associated with the low expression of KLF2 predicts overall shorter survival in breast and prostate cancer (Kaplan–Meier analysis, $P=0.013$ and $P=0.062$, respectively).

also occurred with an enrichment of active transcription marks, such as RNAP-S2 occupancy and the trimethylation of lysine 4 of histone H3 (Figure 3C).

From the point of view of cellular growth, KLF2 also had the expected features of a tumor suppressor gene. KLF2-transfected U2OS cells showed a marked reduction of proliferation determined by both the MTT (Figure 3D) and colony-formation (Figure 3D) assays. Conversely, stable depletion of KLF2 by shRNA in the non-transformed MRC5 fibroblast cells increased cell viability as assessed by the MTT assay (Figure 3E). We also wished to investigate the contribution of KLF2 repression to the overall tumorigenic phenotype conferred by EZH2. To do this, we first depleted EZH2 by RNAi in U2OS cells (Supplementary Figure 5) and observed that the induced diminished levels of EZH2 were associated with a lower level of cell proliferation, as determined by the MTT assay (Supplementary Figure 5). This finding was consistent with those of previous reports (Varambally *et al*., 2002; Bracken *et al*., 2003). As also described above, EZH2–RNAi caused KLF2 upregulation (Supplementary Figure 5). We proceeded to knock down KLF2 by RNAi in the EZH2-depleted cells to investigate whether the loss of KLF2 was able to 'rescue' partially the oncogenic phenotype mediated by EZH2. We observed that the double RNAi against EZH2 and KLF2 (EZH2-/KLF2-cells) gave rise to cells with a higher proliferation rate than those with single EZH2 depletion (Supplementary Figure 5). Thus, KLF2 transcriptional silencing is an important step in the proliferation pathways mediated by the EZH2 oncogene.

We extended the study of the KLF2 growth-inhibitory role to *in vivo* mouse models. Athymic (nu/nu) mice, aged 4–5 weeks, were used for tumor xenograft models. The experimental design was approved by the Bellvitge Biomedical Research Institute Ethical Board. The mice were killed 30 days after injection and tumors were excised and weighed, while the mean volume of tumors ± standard error of the mean (s.e.m.) was also calculated. The subcutaneous injection of 3×10^6 U2OS cells in nude mice demonstrated that KLF2-transfected cells (U2OS-pCMV-KLF2) developed significantly smaller tumors than empty vector-transfected U2OS cells (Student's *t*-test, $P = 0.028$) (Figure 4a). The characterization of the spreading potential was developed by tail-vein injection in the mice of 1×10^6 U2OS cells suspended in 0.2 ml phosphate-buffered saline, and the survival rate at 40 days was analyzed by the Kaplan–Meier method. U2OS-pCMV-KLF2-transfected cells had a significantly lower mortality rate than U2OS empty vector-transfected cells ($P < 0.001$) (Figure 4b). Depletion of EZH2 by shRNA had a similar effect in the reduction of mortality (Figure 4b).

Finally, we decided to translate part of these findings to the context of human primary tumors. We assessed EZH2 and KLF2 expression by immunostaining in clinical tumor samples using a cancer tissue microarray that includes prostate and breast cancer tissue samples with clinical data (SuperBioChips Laboratories, Seoul, Korea). Supplementary Table 1 summarizes the EZH2/KLF2 expression and clinical data from each individual prostate ($n = 40$) and breast ($n = 15$) cancer case. We observed that KLF2 expression was inversely associated with EZH2 expression in prostate (Pearson's correlation coefficient $r^2 = 0.32$, $P < 0.05$) and breast (Pearson's correlation coefficient $r^2 = 0.57$, $P < 0.05$) tumors (Figure 4c). Most importantly, the comparison of the expression data against the clinicopathological values showed that the combination of low KLF2 and high EZH2 expression was associated with shorter overall survival in breast and prostate cancer (Kaplan–Meier analysis, $P = 0.013$ and 0.062, respectively) (Figure 4d).

Overall, our data indicate that KLF2 undergoes transcriptional silencing in human tumorigenesis by the direct repression of an oncogenic Polycomb protein, the histone methyltransferase EZH2. The EZH2-mediated inactivation of KLF2 blocks the tumor-suppressor features of the KLF2 protein, such as its pro-apoptotic and cell cycle-inhibitory capacities, mediated by p15/CDKN2B and p21/CDKN1A, and its growth-inhibitory features demonstrated in cellular and animal models. Most importantly, the EZH2-mediated loss of KLF2 predicts a poor outcome in human malignancies.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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ORIGINAL ARTICLE

Effect of graft sources on allogeneic hematopoietic stem cell transplantation outcome in adults with chronic myeloid leukemia in the era of tyrosine kinase inhibitors: a Japanese Society of Hematopoietic Cell Transplantation retrospective analysis

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Abstract We retrospectively compared transplant outcomes for related bone marrow transplantation (rBMT), related peripheral blood stem cell transplantation (rPBSCT), unrelated bone marrow transplantation (uBMT), and unrelated cord blood transplantation (CBT) in 1,062 patients with chronic myeloid leukemia (CML) aged 20 years or over between January 1, 2000 and December 31, 2009 in Japan. The disease status was as follows: chronic phase 1 (CP1, $n = 531$), CP 2 or later including accelerated phase (CP2-AP, $n = 342$) and blastic crisis (BC, $n = 189$). Graft sources (GS) were rBMT ($n = 205$), uBMT ($n = 507$), rPBSCT ($n = 226$) or CBT ($n = 124$). In multivariate analysis in CP1, lower overall survival (OS) (relative risk [RR]: 6.01, 95 % confidence interval [CI]: 1.20–29.97, $P = 0.029$) and leukemia-free survival (LFS) (RR: 4.26, 95 % CI: 1.24–14.62, $P = 0.021$) were observed in uBMT compared with those in rBMT. For patients in the advanced phase of CML beyond CP1, GS had no significant impact on OS or LFS. Our results support the use of rBMT for adults with CML in CP1, but in contrast to previous reports, the superiority of rPBSCT in advanced stage of CML was not confirmed in our cohorts.

Keywords Chronic myeloid leukemia · Allogeneic hematopoietic stem cell transplantation · Graft sources

On behalf of Choric Myeloid Leukemia Working Group of the Japan Society for Hematopoietic Cell Transplantation.

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Introduction

According to the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the number of transplants reported annually for the treatment of CML was 306 in 2000, but drastically dropped to 46 transplants in the year 2009. Unsurprisingly, the drop in transplant activity was observed in Japan after imatinib (IM) became available as an experimental drug in 2000 and subsequently as a frontline treatment for CML in 2001. Thus, the excellent outcomes demonstrated by tyrosine kinase inhibitors (TKIs) argue against the use of allogeneic hematopoietic stem cell transplantation (allo-HSCT) as an upfront therapy for CML in CP1; allo-HSCT is currently recommended for patients with a T315I mutation, or who failed TKIs and progress to advanced phase disease [1–6]. Moreover, the newly launched third generation TKI, ponatinib, having a unique binding mechanism allowing inhibition of BCR-ABL kinases, including those with the T315I mutation may further narrow the range of transplant indication [7, 8]. Therefore, those CML patients who undergo allo-HSCT represent a selection of high-risk patients due to more advanced disease with high rates of accelerated or blast phase. To improve transplant outcomes, comprehensive approaches in transplant strategies including timing, choice of conditioning and GS, maintenance therapy might be needed for those CML patients being selected nowadays for allo-HSCT. The main purpose of this study was to analyze the impact of GS on transplant outcome for patients with CML in the era of TKIs, particularly the role of GS in each disease status. We also clarified the prognostic factors for transplant outcomes in each disease status. We herein report our analysis of 1,062 patients, whose complete registry-based clinical data which were provided by the JSHCT.

Patients and methods

Patients

Data on a total of 1,143 patients of at least 20 years of age who had undergone allogeneic bone marrow, peripheral blood, or cord blood transplantation for CML between January 1, 2000 and December 31, 2009 were initially collected through the Transplant Registry Unified Management Program (TRUMP). Eighty-one patients were excluded from the analysis, because one or two critical data such as alive, relapse, and engraftment status with or without date of onset were missing. Other missing data were dealt as missing data in the study and the analysis numbers in each variable were described, respectively. This included data from the Japan Cord Blood Bank Network (JCBBN), the Japan Marrow Donor Program (JMDP), and JSHCT. These are the 3 largest allo-HSCT registries in Japan, and their roles have been described previously [9]. The study was approved by the data management committees of JSHCT, as well as by the ethical committee of Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital (Tokyo, Japan), where this study was organized.

Statistical analysis

The outcome endpoints were neutrophil recovery, platelet recovery, acute and chronic GVHD, relapse, transplantation-related mortality (TRM), overall survival (OS), and leukemia-free survival (LFS). The definitions of the statistical models used were in accordance with the statistical guidelines of the European Group for Blood and Marrow Transplantation (EBMT) (http://www.ebmt.org/1WhatisEBMT/whatisebmt2.html). Neutrophil recovery was defined by an absolute neutrophil count (ANC) of at least 0.5×10^9/L for 3 consecutive days, with the first day considered as the recovery day. Platelet recovery was defined by a non-transfused platelet count of at least 20×10^9/L for 3 consecutive days. Deaths occurring before day 90 or day 180 were considered as competing risks for neutrophil or platelet recovery, respectively. The graft failure rate for neutrophils was calculated for patients living without relapse for more than 30 days. Acute and chronic GVHD were diagnosed and graded at each center according to the standard criteria [10–12]. Relapse was defined on the basis of the reappearance of the blast or Philadelphia chromosome (Ph) or *BCR-ABL1* transgene by cytogenetic and/or molecular analysis, including polymerase chain reaction and fluorescence in situ hybridization. TRM was considered a sole cause of non-leukemic deaths occurring after transplantation; OS was defined as the time between transplantation and death due to any cause; LFS was defined as the time interval from allo-HSCT to a first event, either relapse or death, in patients achieving complete remission. HLA antigen disparities were categorised as either GVHD or rejection direction. Low-resolution antigens of HLA-A and HLA-B were identified for all patients by serologic typing or low-resolution molecular typing methods. While, HLA-DRB1 alleles were

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determined by high-resolution molecular typing using the sequence-based HLA typing method. In rBMT, HLA-DRB1 alleles were counted as identical, if the low-resolution antigens of HLA-A, B, and DR were identical. Data on HLA-DRBI allele were not fully available; there were 2 lacking data in CP1, 4 lacking data on CP2-AP and 2 lacking data in BC. Detail of HLA disparity toward either rejection or GVHD are noted in Table 1 and Supplementary Table 1.

Adjusted probabilities of OS and LFS were analyzed using Cox proportional-hazards regression model. The variables used were patients' age at HSCT, patients' sex, body weight at HSCT, time from diagnosis to HSCT, ABO mismatch, conditioning regimen, imatinib administration, kind of GVHD prophylaxis, and year of HSCT. Variables with more than two categories were dichotomized for the final multivariate analyses. Variables were dichotomized as the followings: patient's age at HSCT younger or older than median; patient's body weight at HSCT lighter or heavier than median; time from diagnosis to HSCT <1 year or >1 year. ABO major mismatch or others; myeloablative conditioning regimen or others; cyclosporine-based GVHD prophylaxis regimen or tacrolimus-based; year of HSCT before or after 2004. The endpoints of neutrophil and platelet recovery, acute GVHD and chronic GVHD, relapse and TRM were analyzed using cumulative incidence curves that estimated incidence according to the Fine and Gray models, in which we first used univariate models that contained each of the variables one at a time. Then all variables with a $P < 0.05$ by the likelihood-ratio test were included in a multivariate model.

Cause-specific hazard ratios were estimated with 95 % confidence intervals (CIs). Statistical analysis was performed with the R Foundation statistical computing package, version 2.12.2 (http://www.r-project.org/).

Table 1 Characteristics of patients with CML in CP1, CP2-AP, and BP

	CP1 ($n = 531$)	CP2-AP ($n = 342$)	BP ($n = 189$)
Graft source rBMT/uBMT/rPBSCT/CBT	138/258/125/10	43/176/59/64	24/73/42/50
Gender Male/female	338/193 ($P < 0.001$)	215/127 ($P < 0.001$)	123/66 ($P < 0.001$)
Median age at transplantation (range)	40 (20–67)	43 (21–69)	43 (20–74)
GVHD prophylaxis CyA + MTX/CyA based/FK + MTX/FK based/ others	331/27/144/12/14[a]	148/17/145/19/9[a]	88/22/58/17/2[a]
Pre-transplant IM Yes/no	133/249[b] ($P < 0.001$)	187/108[b] ($P < 0.001$)	94/95 ($P = 0.94$)
Duration from diagnosis to transplantation, months median (range)	12.5 (0.8–169.0)	18.2 (1.6–255.3)	15.5 (2.4–322.7)
Duration from diagnosis to transplantation ≤1 year/> 1 year	248/258[c] ($P = 0.65$)	135/195[c] ($P < 0.001$)	80/100[c] ($P = 0.14$)
Patient's body weight, kg Median (range)	61 (40–104)	60 (34–104)	58.5 (34–96)
Conditioning regimen Myeloablative/reduced intensity	475/53[d] ($P < 0.001$)	289/53 ($P < 0.001$)	161/28 ($P < 0.001$)
Years at transplantation 2000–2004/2005–2009	447/84 ($P < 0.001$)	211/131 ($P < 0.001$)	116/73 ($P < 0.01$)
ABO mismatch No/yes	189/161[e] ($P = 0.13$)	132/156[e] ($P = 0.16$)	64/91[e] ($P = 0.03$)
HLA disparities (rejection direction)[g] 0–1/> 2	510/19[f] ($P < 0.001$)	281/57[f] ($P < 0.001$)	145/42[f] ($P < 0.001$)
HLA disparities (GVHD direction)[g] 0–1/> 2	507/22[f] ($P < 0.001$)	285/53[f] ($P < 0.001$)	140/47[f] ($P < 0.001$)

CP chronic phase, *AP* accelerated phase, *BP* blastic phase, *rBMT* related bone marrow transplantation, *rPBSCT* related peripheral blood stem cell transplantation, *uBMT* unrelated bone marrow transplantation, *CBT* unrelated cord blood transplantation, *GVHD* graft-versus-host disease, *CyA* cyclosporine, *MTX* methotrexate, *FK* tacrolimus, *IM* imatinib mesylate, *HLA* human leukocyte antigen

[a] Data on GVHD prophylaxis were not fully available; there were 3 missing data in CP data, 4 missing data on CP2-AP and 2 missing data in BC

[b] Data on pre-transplant imatinib administration were not fully available; 149 data and 47 data were not retrieved in CP1 and in CP2-AP, respectively

[c] Loss of data on duration from diagnosis to transplantation (≤ 1 year/> 1 year) was noted; 25 data in CP, 12 data in CP2-AP, and 9 data in BP were not retrieved

[d] Three data regarding conditioning regimen in CP were not retrieved

[e] Loss of data on ABO mismatch was noted; 181 data in CP, 54 data in CP2-AP, and 34 data in BP were not retrieved

[f] Data on HLA-DRBI allele were not fully available; there were 2 lacking data in CP, 4 lacking data on CP2-AP and 2 lacking data in BC

[g] More detail of HLA disparity toward either rejection or GVHD is noted in supplementary Table 1

Results

Patient characteristics

Of 1,062 patients (676 men, 386 women; median age, 41 years; range, 20–74), 414 patients (39 %) had a clear history of pre-transplant IM use. Disease status was as follows: CP1 ($n = 531$), CP2-AP ($n = 342$) and BC ($n = 189$). GS were related rBMT ($n = 205$), uBMT ($n = 507$), rPBSCT ($n = 226$) and CBT ($n = 124$). The unrelated PBSCT has not been allowed in Japan until 2012 and, therefore, our data included only unrelated BMT, not PBSCT. In addition, during the study period, there were no related CBTs at all. The other variables, including GVHD prophylaxis, pre-transplant IM, body weight at allo-HSCT, duration from diagnosis to transplant, conditioning intensity, years at transplantation (2000–2004 vs. 2005–2009), ABO mismatch, HLA mismatch in either GVHD or rejection direction, are shown in Table 1.

Overall survival and leukemia-free survival

The median follow-up period was 914 days after transplantation (range 2–3,902) and 1,914 days after diagnosis (range 29–9,120). Three-year OS was 70.6 % (95 % CI, 66.8-74.7 %) for patients in CP1 at the time of transplantation, 58.9 % (95 % CI, 53.7–64.7 %) for those with CP2-AP, and 26.9 % (95 % CI, 20.9–34.6 %) for those in BC. The probability of 3-year LFS for patients in CP1, CP2-AP and BC was 64.6 % (95 % CI, 60.4–68.6 %), 46.1 % (95 % CI, 40.9–51.9 %) and 19.2 % (95 % CI, 14.1–26.1 %), respectively (data not shown).

OS and LFS according to GS in CP1, CP2-AP, and BC are shown in Fig. 1a–c, and d–f, respectively. In view of OS and LFS according to GS, 3-year OS after rBMT, rPBSCT, uBMT, and CBT in CP1 was 84.4, 70.0, 64.4, and 48.0 %, respectively (Fig. 1a). Three-year LFS after rBMT, rPBSCT, uBMT, and CBT in CP1 was 76.3, 64.3, 59.3, and 30 %, respectively (Fig. 2d). Multivariate analysis for OS identified the following factors as adverse prognostic factors for

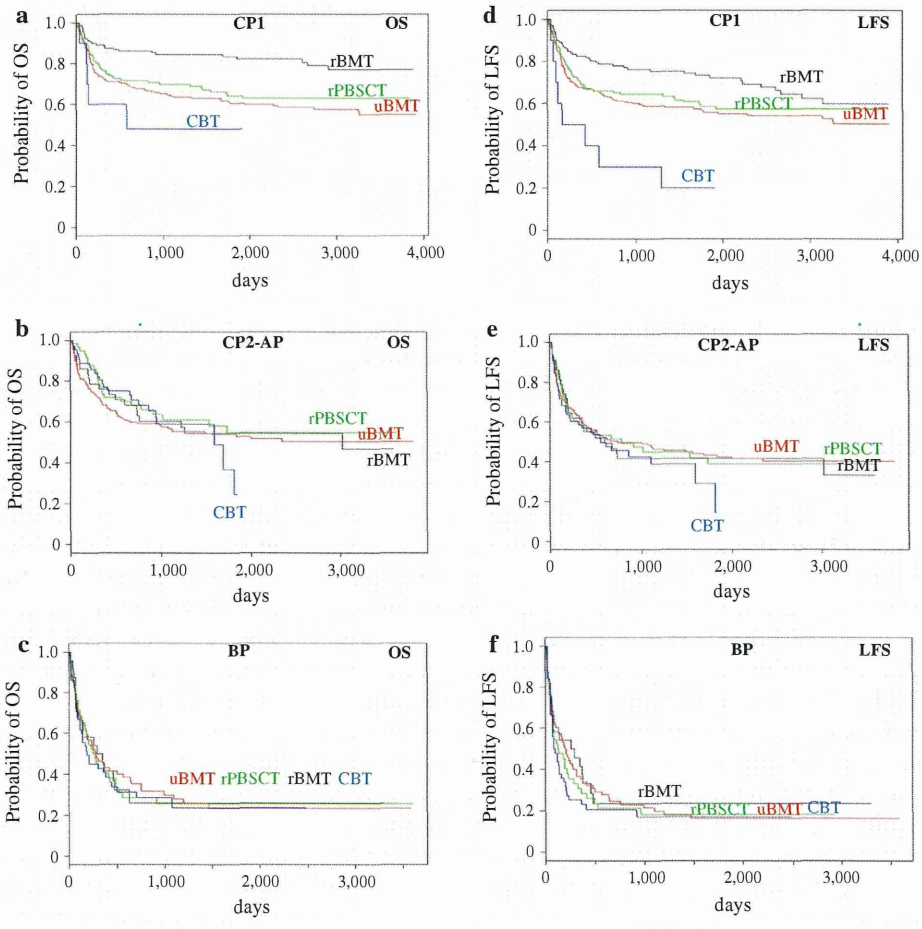

Fig. 1 Kaplan–Meier estimate of overall survival (OS) for patients in CP1 (**a**), CP2-AP (**b**) and BC (**c**); and leukemia-free survival (LFS) for patients in CP1 (**d**), CP2-AP (**e**) and BC (**f**)

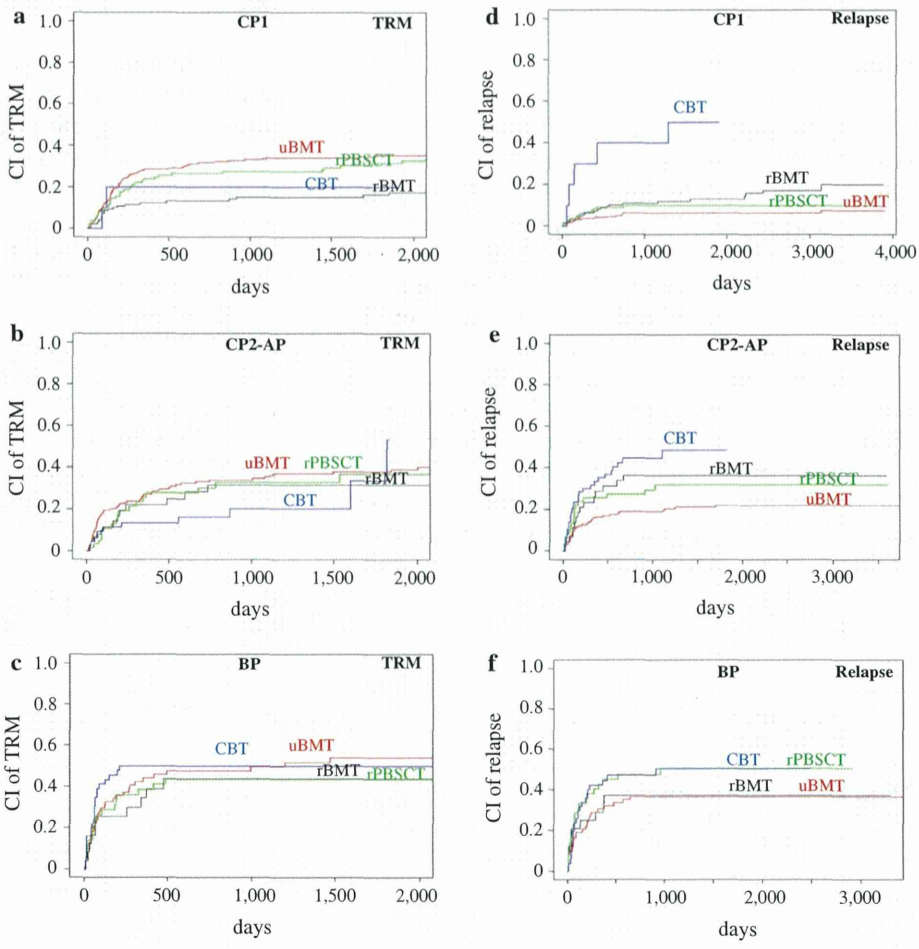

Fig. 2 The cumulative incidence of transplantation-related mortality (TRM) for patients in CP1 (**a**), CP2-AP (**b**) and BC (**c**); and relapse for patients in CP1 (**d**), CP2-AP (**e**) and BC (**f**)

patients in CP1: older age (>median age, 40 years: HR 1.67, 95 % CI, 1.15–2.41, $P = 0.007$), ABO mismatch (HR 1.44, 95 % CI, 1.003–2.06, $P = 0.048$) (Table 2), and uBMT (RR 6.01, 95 % CI, 1.20–29.97, $P = 0.029$) (Table 3). In CP2-AP, older age (> median age, 43 years: HR 1.74, 95 % CI, 1.25–2.43, $P < 0.001$) was the only factor an adverse prognostic factor (Table 2). In BC, pre-transplant IM (HR 0.61, 95 % CI, 0.49–0.89, $P = 0.011$) was the only factor for better OS (Table 2). Concerning LFS, multivariate analysis showed that uBMT (RR 4.26, 95 % CI, 1.24–14.62, $P = 0.021$) and older age (>median age, 40 years: HR 1.43, 95 % CI, 1.02–1.99, $P = 0.038$) were adverse risk factors in CP1 (Table 2, 3). For patients in CP2-AP and BC, no significant factor for OS or LFS was found. Thus, for patients in CP1, GS could have a significant impact on survival outcomes. While, for patients in the advanced phase of CML of beyond CP1, GS could have no significant impact on OS or LFS (Table 3).

TRM and relapse

The 1-year cumulative TRM rate by disease stage was 23.1 % (95 % CI, 19.5–26.7 %) in CP1, 24.2 % (95 % CI, 19.5–28.9 %) in CP2-AP, and 43.2 % (95 % CI, 35.9–50.5 %) in BC. TRM by GS is shown in Fig. 2a–c. The TRM rate appeared low in rBMT compared with uBMT or rPBSCT in CP1 (Fig. 2a). Multivariate analysis showed that uBMT (RR 2.49, 95 % CI 1.02–6.10, $P = 0.046$) and older age (>median age, 40 years: HR 1.69, 95 % CI, 1.19–2.39, $P = 0.003$) were factors associated with a significantly increased risk of TRM in CP1 (Table 2, 3).

The 3-year cumulative relapse rate by disease stage was 9.0 % (95 % CI, 3.9–7.9 %) in CP1, 28.2 % (95 % CI, 23.3–33.1 %) in CP2-AP, and 43.6 % (95 % CI, 36.3–50.9 %) in BC. Relapse rate by GS is demonstrated in Fig. 2d–f. For patients in CP1, the relapse rate after CBT appeared to be higher than that after other GS (Fig. 2d). In multivariate analysis by the effect of GS in CP1, CBT (RR

Table 2 Multivariate analysis of risk factors for the main outcomes after allo-HSCT for CML in CP1, CP2-AP, and BP

Main outcomes	Factors	CP1				CP2-AP				BP			
		Factors	HR	(95 % CI)	*P* value	Factors	HR	(95 % CI)	*P* value	Factors	HR	(95 % CI)	*P* value
OS	Age	≤40	1			≤43	1						
		>40	1.67	1.15–2.41	0.007	>43	1.74	1.25–2.43	< 0.001				
	ABO mismatch	No	1										
		Yes	1.44	1.003–2.06	0.048								
	Pre-transplant IM									No	1		
										Yes	0.61	0.41–0.89	0.011
LFS	Age	≤40	1										
		>40	1.43	1.02–1.99	0.038								
TRM	Age	≤40	1										
		>40	1.69	1.19–2.39	0.003								
Relapse	HLA mismatch (rejection)									0, 1	1		
										≥2	1.7	1.04–2.76	0.033
	HLA mismatch (GVHD)					0, 1	1						
						≥2	3.57	1.55–8.21	0.003				
Acute GVHD (all grades[a])	Pre-transplant IM	No	1										
		Yes	0.75	0.57–0.99	0.04								
	BW					≤60 kg	1						
						>60 kg	1.35	1.01–1.82	0.045				
Acute GVHD	BW					≤60 kg	1						
(≥grade 2)						> 60 kg	1.53	1.05–2.24	0.028				
Chronic GVHD (extensive[b])	BW					≤60 kg	1						
						>60 kg	1.75	1.06–2.73	0.028	0			

OS overall survival, *LFS* leukemia-free survival, *TRM* transplantation-related mortality, *ANC* absolute neutrophil count, *GVHD* graft-versus-host disease, *IM* imatinib, *HLA* human leukocyte antigen, *BW* body weight, *HR* hazard ratio, *CI* confidence interval, *CP* chronic phase, *AP* accelerated phase, *BP* blastic phase, *imatinib* imatinib mesylate

[a] Overall grade of acute GVHD assigned according to the Center for International Blood and Marrow Transplant Research (CIBMTR) severity index

[b] Chronic GVHD was graded as limited or extensive based on the Seattle criteria

Table 3 Impact of graft sources on main outcomes after allo-HSCT for CML in CP1, CP2-AP, and BP

Main outcomes	Graft sources	CP1			CP2-AP			BP		
		RR	(95 % CI)	*p* value	RR	(95 % CI)	*p* value	RR	(95 % CI)	*p* value
OS	rBMT	1.00			1.00			1.00		
	uBMT	6.01	(1.20–29.97)	0.029	1.12	(0.33–3.79)	0.851	>99	(0.00–99.99)	0.999
	rPBSCT	1.76	(0.77–4.04)	0.180	0.84	(0.21–3.43)	0.809	1.13	(0.56–2.30)	0.727
	CBT	1.00	(0.00–99.99)	1.000	NA	NA	NA	NA	NA	NA
LFS	rBMT	1.00			1.00			1.00		
	uBMT	4.26	(1.24–14.62)	0.021	1.61	(0.55–4.74)	0.383	0.00	(0–99.99)	0.999
	rPBSCT	1.72	(0.95–3.11)	0.073	0.42	(0.14–1.31)	0.135	0.67	(0.31–1.44)	0.299
	CBT	1.00	(0.00–99.99)	1.000	NA	NA	NA	NA	NA	NA
TRM	rBMT	1.00			1.00			1.00		
	uBMT	2.49	(1.02–6.10)	0.046	1.36	(0.60–3.09)	0.47	2.71	(0.74–9.96)	0.13
	rPBSCT	1.03	(0.52–2.07)	0.93	0.94	(0.52–1.70)	0.83	1.43	(0.64–3.22)	0.39
	CBT	0.33	(0.04–2.63)	0.29	0.98	(0.60–1.60)	0.94	1.26	(0.82–1.92)	0.29
Relapse	rBMT	1.00			1.00			1.00		
	uBMT	0.33	(0.12–0.95)	0.041	0.66	(0.29–1.55)	0.34	2.23	(0.28–17.61)	0.45
	rPBSCT	1.13	(0.62–2.07)	0.68	1.17	(0.64–2.14)	0.6	1.06	(0.44–2.54)	0.9
	CBT	25.16	(1.76–369.10)	0.018	1.15	(0.74–1.80)	0.53	0.77	(0.39–1.60)	0.49
ANC recovery	rBMT	1.00			1.00			1.00		
	uBMT	0.82	(0.55–1.23)	0.35	0.83	(0.53–1.31)	0.43	0.58	(0.27–1.26)	0.17
	rPBSCT	1.31	(1.02–1.69)	0.036	1.2	(0.90–1.59)	0.21	0.91	(0.33–2.52)	0.86
	CBT	2	(0.67-5.98)	0.22	0.53	(0.42–0.67)	<0.001	0.55	(0.37–0.82)	0.003
Platelet recovery	rBMT	1.00			1.00			1.00		
	uBMT	0.75	(0.46–1.21)	0.24	0.89	(0.51–1.56)	0.68	0.21	(0.07–0.61)	0.0039
	rPBSCT	0.93	(0.69–1.26)	0.65	0.91	(0.61–1.35)	0.63	0.67	(0.28–1.57)	0.35
	CBT	1.07	(0.35–3.28)	0.9	0.78	(0.62–0.99)	0.049	0.44	(0.26–0.74)	0.0018
Acute GVHD (all grades[a])	rBMT	1.00			1.00			1.00		
	uBMT	3.35	(1.50–6.22)	<0.001	1.67	(0.92–3.02)	0.09	1.22	(0.46–3.25)	0.69
	rPBSCT	1.49	(0.94–2.37)	0.091	0.86	(0.51–1.44)	0.56	0.94	(0.32–2.73)	0.91
	CBT	1.67	(0.68–4.11)	0.26	0.76	(0.58–1.01)	0.054	1.05	(0.56–1.96)	0.87
Acute GVHD (≥grade 2)	rBMT	1.00			1.00			1.00		
	uBMT	4.28	(1.92–9.53)	<0.001	2.14	(0.93–4.94)	0.075	1.34	(0.39–4.61)	0.65
	rPBSCT	1.5	(0.82–2.72)	0.19	1.53	(0.82–2.86)	0.18	2.23	(0.36–1.39)	0.39
	CBT	1.00	(0.00–99.99)	1.000	0.84	(0.58–1.22)	0.36	1.45	(0.55–3.81)	0.45
Chronic GVHD	rBMT	1.00			1.00			1.00		
	uBMT	0.95	(0.53–1.70)	0.86	1.1	(0.45–2.68)	0.84	0.27	(0.06–1.33)	0.11
	rPBSCT	1.37	(0.97–1.92)	0.075	1.24	(0.70–2.19)	0.47	0.84	(0.22–3.20)	0.8
	CBT	8.52	(0.64–11.43)	0.11	0.8	(0.52–1.25)	0.33	0.73	(0.32–1.66)	0.46
Chronic GVHD (extensive[b])	rBMT	1.00			1.00			1.00		
	uBMT	1	(0.49–2.04)	1	0.84	(0.33–2.15)	0.72	0.69	(0.14–3.46)	0.65
	rPBSCT	1.31	(0.87–1.96)	0.19	1.19	(0.60–2.34	0.62	1.08	(0.27–4.24)	0.92
	CBT	6.61	(0.22–200.8)	0.28	0.63	(0.36–1.09)	0.097	0.77	(0.31–1.88)	0.56

OS overall survival, *LFS* leukemia-free survival, *TRM* transplantation-related mortality, *ANC* absolute neutrophil count, *GVHD* graft-versus-host disease, *RR* relative risk, *CI* confidence interval, *CP* chronic phase, *AP* accelerated phase, *BP* blastic phase, *rBMT* related bone marrow transplantation, *rPBSCT* related peripheral blood stem cell transplantation, *uBMT* unrelated bone marrow transplantation, *CBT* unrelated cord blood transplantation, *NA* not available

[a] Overall grade of acute GVHD assigned according to the Center for International Blood and Marrow Transplant Research (CIBMTR) severity index

[b] Chronic GVHD was graded as limited or extensive based on the Seattle criteria

25.16, 95 % CI 1.76–369.10, $P = 0.018$) showed higher relapse, while uBMT (RR 0.33, 95 % CI 0.12–0.95, $P = 0.041$) was lower relapse compared with those in rBMT (Table 3).

Engraftment

The cumulative neutrophil recovery rate on day 90 was 97.5 % (95 % CI, 96.1–98.9 %) in CP1, 93.2 % (95 % CI, 90.5–95.9 %) in CP2-AP, and 82.3 % (95 % CI, 76.8–87.8 %) in BC. On day 180, the cumulative platelet recovery rate, as indicated by more than 2×10^{10}/L of platelets in blood, was 91.9 % (95 % CI, 89.5–94.3 %) in CP1, 85.1 % (95 % CI, 81.2–89.0 %) in CP2-AP, and 67.2 % (95 % CI, 60.3–74.1 %) in BC. Note that the neutrophil recovery and platelet recovery rates were lower after CBT, especially in patients in the advanced phase; i.e., neutrophil recovery in CBT: 90 % in CP1, 79.4 % in CP2-AP, and 64.0 % in BC; platelet recovery after CBT: 90.0 % in CP1, 72.5 % in CP2-AP, and 52.0 % in BC (Fig. 3a–f). Multivariate analysis showed that rPBSCT (RR 1.31, 95 % CI 1.02–1.69, $P = 0.0396$ was a significant factor for early neutrophil recovery in CP1. While, CBT (RR 0.53, 95 % CI 0.42–0.67, $P < 0.001$) was a significant factor for delayed neutrophil recovery in CP2-AP (Table 3). The factor statistically associated with delayed platelet recovery was CBT in CP2-AP (RR 0.78, 95 % CI 0.62–0.99, $P = 0.0049$) and in BC (RR 0.44, 95 % CI 0.26–0.74, $P = 0.0018$). Unrelated BMT (RR 0.21, 95 % CI 0.07–0.61, $P = 0.0039$) was also a significant factor for delayed platelet recovery in BC (Table 3).

Acute and chronic GVHD

The cumulative incidence of acute GVHD at all grades before day 100 was 62.8 % (95 % CI, 58.6–67.0 %) in CP1, 63.5 % (95 % CI, 58.2–58.8 %) in CP2-AP, and 68.6 % (95 % CI, 61.3–74.9 %) in BC. Patients who underwent uBMT showed a higher incidence of acute GVHD (all grades) in CP1 and CP2-AP (Fig. 4a, b). This association was confirmed by multivariate analysis; uBMT (RR 3.35, 95 % CI 1.50–6.22, $P < 0.001$) was a significant factor in CP1 (Table 3). Pre-transplant IM (HR 0.75, 95 % CI 0.57–0.99, $P = 0.04$) was a significant risk factor for acute GVHD (all grades) in CP1 (Table 2). Focusing exclusively on grade II or higher acute GVHD, uBMT (RR 4.28, 95 % CI 1.92–9.53, $P < 0.001$) (Table 3) was a significant risk factor in CP1 (Table 2). For patients in CP2-AP, body weight (>60 kg) was a factor significantly associated with increased risk of aGVHD (all grade; RR 1.35, 95 % CI, 1.01–1.82, $P = 0.045$, grade II or higher grade; RR 1.53, 95 % CI, 1.05–2.24, $P = 0.028$) (Table 2).

The cumulative incidence of chronic GVHD among evaluable patients who survived at least 100 days after allo-HSCT was 49.4 % (95 % CI, 44.7–54.1 %) in CP1, 42.2 % (95 % CI, 36.4–48.0 %) in CP2-AP, and 37.8 % (95 %CI, 30.0–45.6 %) in BC. For patients in CP1, rPBSCT showed a higher incidence of chronic GVHD (71.4 %), which was compared to other GS (Fig. 4d); however, this significant association was not confirmed in multivariate analysis (rPBSCT: RR 1.37 95 % CI 0.97–1.92, $P = 0.075$). For patients in CP2-AP and BC, chronic GVHD after CBT occurred at rates of 23.1 and 23.8 %, respectively, which were apparently lower than that of other GS (Fig. 4e, f), but these statistical associations were not also confirmed by multivariate analysis in CP2-AP or BC (Table 3). Concerning extensive chronic GVHD, multivariate analysis showed the significant association between body weight (>60 kg; RR 1.75, 95 % CI, 1.06–2.73, $P = 0.028$) and chronic GVHD in CP2-AP (Table 2).

Discussion

Our study reviewed 1,062 Japanese adult patients who underwent allo-HSCT during the past decade (2000–2009); thus, our cohort reflects the current use and results of allo-HSCT for CML in Japan. Moreover, the TRUMP database offers the advantage of a large number of patients with extensive data, which permits multivariate analysis. The 3-year OS was 70.6 % for patients in CP1, and the probability of 3-year LFS for patients in CP1 was 64.6 %. These survival data for patients in CP1 were comparable to those reported by others [12]. Based on the report from the EBMT, which included 13,416 CML patients and was apparently the largest CML transplant database including the 3 times cohorts (i.e., 1980–1990, 1991–1999, 2000–2003), the probability of OS at 2 years for patients transplanted in CP1 from an HLA-identical sibling was 74 %, with a cumulative incidence of TRM at 2 years of 22 % and of relapse of 18 % among the most recent cohort transplanted between 2000 and 2003 ($n = 3{,}018$) [13]. The Center for International Blood and Marrow Transplant Research (CIBMTR) recently reported the transplant outcomes of 449 patients with advanced phase CML; the disease-free survival rates remained as low as 35–40 % for CP2, 26–7 % for AP, and 8–11 % for BC [14]. Our series including 432 cases of CP2-AP and 189 cases of BC showed similar survival rates, as the probabilities of 3-year LFS in CP2-AP and BC were 46.1 and 19.2 %, respectively.

Our primary object in this study was to assess the clinical impact of GS according to each disease status. Our study results revealed that the patients in CP1 who were

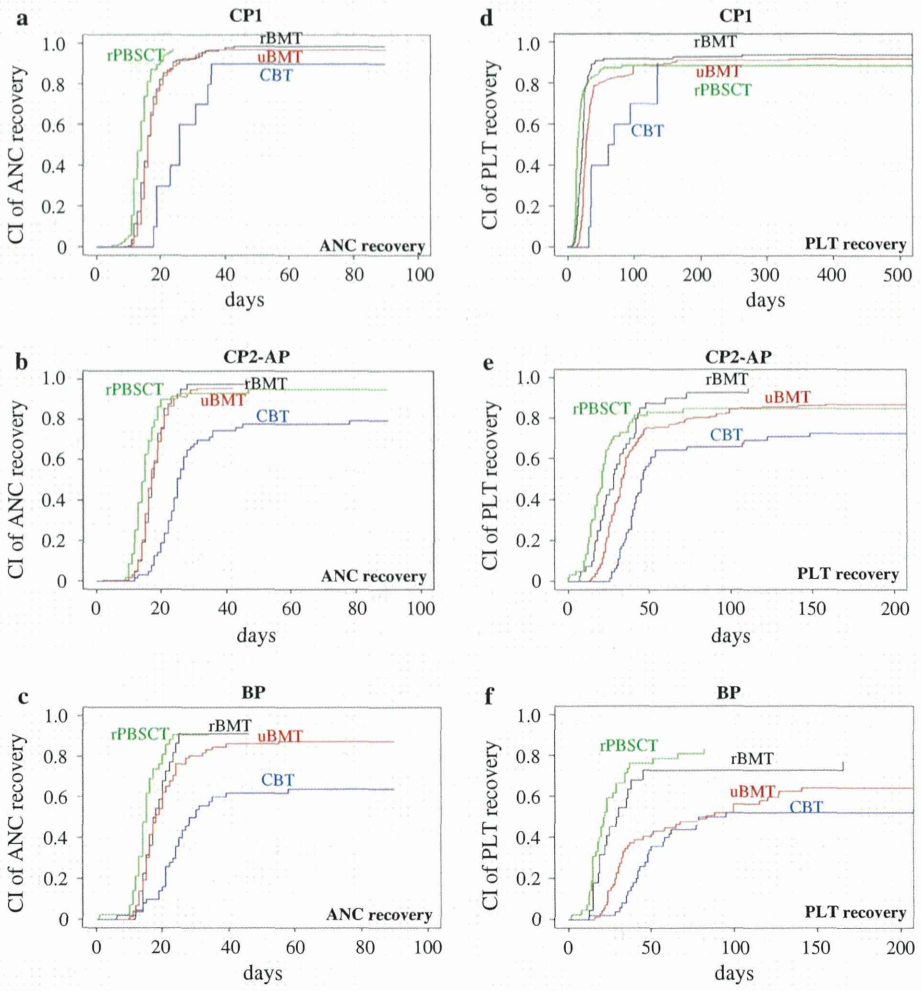

Fig. 3 The cumulative incidence of absolute neutrophil count (ANC) recovery for patients in CP1 (**a**), CP2-AP (**b**) and BC (**c**); and platelet (PLT) recovery for patients in CP1 (**d**), CP2-AP (**e**) and BC (**f**)

treated by rBMT showed a better 3-year OS (84.4 %) with a lower 1-year cumulative incidence of TRM, but the 3-year LFS and relapse rates were similar between patients receiving rBMT and patients receiving rPBSCT. These data were essentially in line with previous reports in which the CIBMTR reported the data of CML patients undergoing rPBSCT or rBMT in CP1; the 1-year LFS and relapse rates were similar for patients receiving rBMT or rPBSCT [14]. We also assessed the clinical impact of GS in CP2-AP; our results showed that there were no significant differences in OS or LFS between GS, despite lower probabilities of relapse after uBMT and lower probabilities of TRM after CBT. These results differ from the IBMTR reports in that for patients in CP2 or AP, rPBSCT was associated with a lower incidence of treatment failure and a higher probability of LFS at 1 year [15]. Regarding GVHD, a recent prospective randomized trial showed a trend toward a higher incidence of chronic GVHD after rPBSCT (59 % after rPBSCT vs. 40 % after rBMT, $P = 0.11$) for patients in CP1 [16]. Our results may confirm this report; although multivariate analysis in our study showed that rPBSCT (RR 1.37 95 % CI 0.97–1.92, $P = 0.075$) was not a significant risk factor for developing chronic GVHD (Table 3), rPBSCT showed a higher incidence of chronic GVHD (71.4 %), which was compared to other GS in CP1 (Fig. 4d).

Several investigators have addressed the clinical impact of pre-transplant IM on post-transplant outcomes for CML [14, 17–20]. The CIBMTR data demonstrated that pre-transplant IM was associated with better survival, but revealed no statistically significant differences in TRM, relapse, and LFS for patients in CP1 [17]. Among patients transplanted in the more advanced phases beyond CP1, pre-transplant IM was not associated with TRM, relapse, LFS, OS, or acute GVHD [17]. In contrast to these studies, our analysis showed that pre-transplant IM was significantly associated with better OS for patients in BC. In addition, multivariate analysis found pre-transplant IM was a

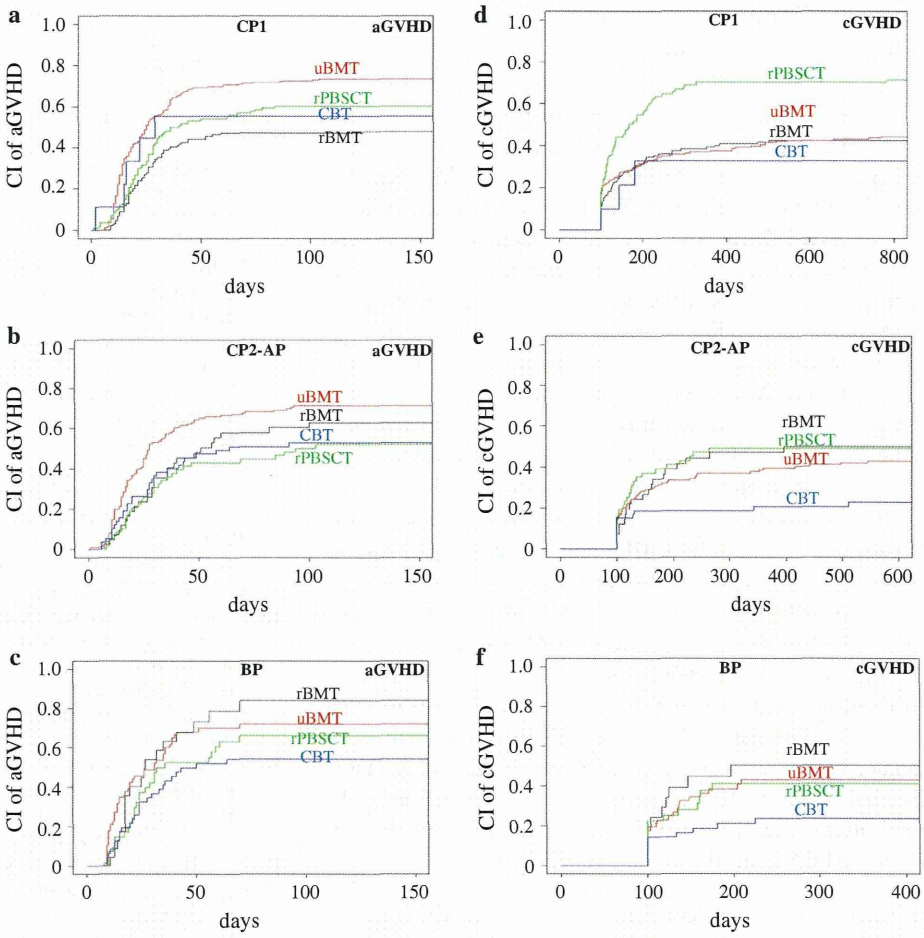

Fig. 4 The cumulative incidence of acute GVHD at all grades for patients in CP1 (**a**), CP2-AP (**b**) and BC (**c**); and chronic GVHD at all grades for patients in CP1 (**d**), CP2-AP (**e**) and BC (**f**)

significant factor associated with acute GVHD (>grade II) in CP1 (data not shown). Despite the study in the era of TKI, half of patients were in CP1, and 61 % of patients underwent allo-HSCT without use of pre-transplant TKI in this study. We should interpret these findings with utmost caution. We assume that most patients had already initiated the conventional treatment but could not reach a new, but expensive IM treatment before allo-HSCT, as a reason for these findings. Moreover, the findings that the number of patients in CP1 underwent allo-HSCT was 447 in the early period of IM from 2000 to 2004 and only 84 from 2005 to 2009 might support our assumption. Deininger et al. reported an effect of pre-transplant IM in their study that included 70 cases of CML and 21 cases of Ph (+) acute lymphoid leukemia. These investigators compared the outcomes with historical controls identified in the EBMT database [21], and observed a trend towards higher relapse mortality and significantly less chronic GVHD in patients with pre-transplant IM (OR = 0.44, $P = 0.027$). Thus, the clinical impact of pre-transplant IM is still a contentious issue; additional studies evaluating the long-term use of IM with a larger number of patients might permit a more refined analysis of the effect of pre-transplant IM.

Although data on clinical outcomes after CBT are conflicting, CBT has apparent advantages over uBMT, including no risk to the donor and ease of availability. Previous reports, mostly from pediatric studies, have shown that, despite higher HLA mismatch, CBT carries a lower risk of acute GVHD and chronic GVHD in comparison with uBMT [22–24]. A recent Japanese retrospective analysis assessing 86 patients, including pediatric patients, disclosed the transplant outcomes of CBT: 2-year OS was 53 %; for patients in CP, AP and BC, the OS rates were 71, 59 and 32 %, respectively [25]. Although our small population with only 10 cases of CBT in CP1 may prohibit drawing meaningful conclusions, a trend of higher relapse and lower TRM, OS and LFS in CP1 was similar to results obtained by previous study groups. Nevertheless, in CP2-AP and BC, transplant outcomes after CBT were comparable to those of other GS,

suggesting CBT as an acceptable alternative option in advanced phases of CML.

As with all retrospective studies, this study had several limitations. Reported data from transplant centers were often incomplete: data on pre-transplant IM, duration from diagnosis to transplantation, and conditioning regimen could not be fully retrieved. The reasons for which patients in CP1 with IM proceeded with transplantation (planned, or IM resistance) or the reasons for delay in proceeding with transplantation in BC were unknown. Information on post-transplant use of TKIs as maintenance therapy or data on the presence of *BCR/ABL1* mutations was also unavailable in our cohort. Moreover, the selection of GS would often be governed by several unmeasured factors, but our data nonetheless provide a clinical basis for current selection of GS for the treatment of CML in the era of TKIs.

In conclusion, this retrospective study evaluated the results of allo-HSCT for CML patients according to disease status and GS. For patients in CP1, rBMT may be the preferred option for better survival, whereas rPBSCT carries a higher risk for chronic GVHD, which could be a major drawback for patients in CP1. In advanced phases, GS had no significant impact on survival, suggesting that CBT is a reasonable alternative therapy when there is no related or unrelated donor available, or when a transplant is needed urgently. In the era of the new-generation TKIs, indications for allo-HSCT and selection of GS for advanced CML need further evaluation.

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Conflict of interest The authors declare no conflict of interest.

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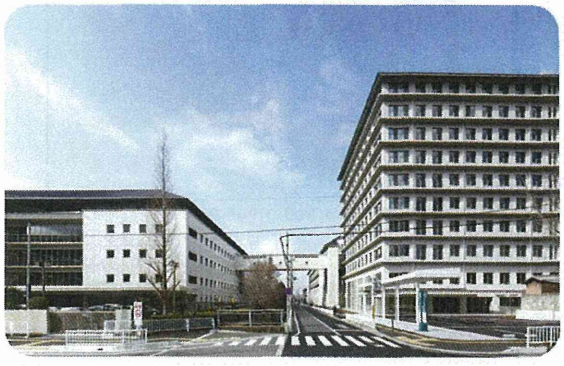

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トランスレーショナルリサーチの重要性

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key words：アカデミア発　橋渡し研究加速ネットワークプログラム　NIH基礎研究　開発型

医薬品開発の動向

2004年に米国食品医薬品局（Food and Drug Administration；FDA）は“Challenge and Opportunity on the Critical Path to New Medical Products”を発出した．この中で，**図1**に示すように医薬品開発への投資額は製薬企業および政府の開発機関である米国立衛生研究所（National Institute of Health；NIH）ともに年々増加しているにもかかわらず，**図2**に示すようにFDAで承認申請が受理される（承認ではない）数が医薬品・生物製剤ともに減少していた．先の白書はこれに対して非常な危機感を示し，規制当局の側から開発を促進する対応策を打ち出していく意図が込められていた．

一方，このころより，製薬業界では「2010年問題」への対応が大きな問題となっていた．これは，売り上げの上位に位置する医薬品の多くが特許切れを迎えるが，それにとって代わる大型の新薬開発が進んでいないため，経営上大きな危機を迎えるというものであった．**表1**は2002年と2012年の医薬品の世界売り上げトップ10を示したものである．2002年は，2位に赤血球造血刺激因子でバイオ製剤であるエリスロポエチン製剤が入っているが，その他は化合物の薬物作用を探求するスクリーニング等により開発された医薬品であった．また，その多くは「2010年問題」に含まれる特許切れを迎えていた．2012年になるとトップ10の医薬品は全て入れ替わっているが，特に注目する点として，6品目が特定の作用機序に関与する分子をターゲットとした分子標的療法薬であり，そのうち5品目が抗体であったことが挙げられる．ヒュミラ®とレミケード®の作用機序は関節リウマチ等の原因であるTNFαの過剰生産に対して抗体でTNFαを阻害することであり，エンブレル®はTNFが結合する受容体とヒト免疫グロブリンのFc部分から構成されており，TNFが細胞表面の受容体と結合することを阻害する．リツキサン®はB細胞性リンパ腫ではリンパ腫細胞表面にCD20が発現していること，ハーセプチン®は乳がん等でHER2が過剰発現している場合が多いことに注目し，悪性細胞で発現している分子に抗体が結合し障害を与えることが作用機序である．固形がんでは血管新生による腫瘍の増殖が認められるが，アバスチン®は血管新生を促進する分子であるVEGFの作用を阻止する抗体として開発された．いずれも基礎研究での発見に注目し，医薬品として抗体あるいは受容体製剤として開発されている．

トランスレーショナルリサーチ（Translational Research；TR）は，基礎研究の成果を初期臨床試験の段階まで臨床応用することが1つの定義である．抗体薬や受容体薬の開発は基礎研究の成果を基になされており，TRの代表といえる．新たな医薬品開発の方策としてTRは重要視されるようになり，各国の医薬品開発

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