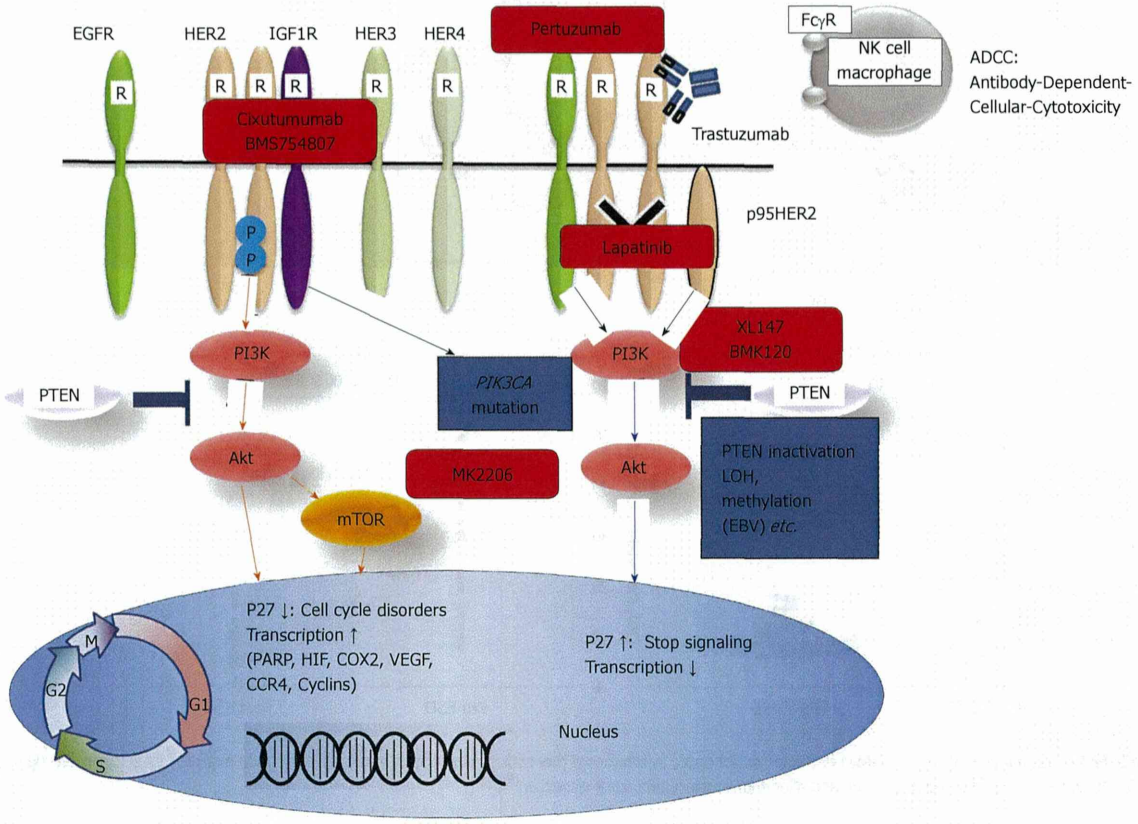

Figure 4 Human epidermal growth receptor family members, the PI3K/Akt pathway, and targeted drugs. HER: Human epidermal growth receptor; NK: Natural killer; IGF1R: α-insulin-like growth factor 1-receptor; EGFR: Epidermal growth factor receptor; PI3K: Phosphatidylinositol-3-kinase; PTEN: Phosphatase and tensin homologue.

GC cells suppressed colony formation. The data suggest that the silencing of Sox17 occurs frequently in early GC and plays a key role in the disease. Gastric wash-based DNA methylation analysis could be useful for the early detection of recurrence following endoscopic resection in early GC patients. Interestingly, the usefulness of gastric wash-based molecular testing for antibiotic resistance in *H. pylori* has also been reported[58]. It will be interesting to analyze gastric washes using NGS.

Anti-HER2 antibody trastuzumab has led to an era of personalized therapy in GC

Trastuzumab is an antibody that targets the HER2 extracellular domain and induces antibody-dependent cellular cytotoxicity and inhibition of the HER2 downstream signals (Figure 4). In the ToGA study, standard chemotherapy regimens (capecitabine plus cisplatin or fluorouracil plus cisplatin) combined with trastuzumab resulted in a longer survival time than standard regimens without trastuzumab in patients with HER2-positive GC[59]. Thus, HER2 expression has become a major concern in GC[60]. HER2 overexpression is observed in 7%-34% of GC cases. Mechanisms of resistance to trastuzumab have been reported in breast cancer. There are various mechanisms underlying trastuzumab resistance, such as alterations of the HER2 structure or surroundings, dysregulation of HER2 downstream signal effectors and interaction of HER2 with other membrane receptors (Figure 4). The PI3K-Akt pathway is one of the main downstream signaling pathways of HER2. It is well known that PIK3CA mutations and PTEN inactivation cause over-activation of a downstream signal without activation of an upstream signal. The frequencies of PIK3CA mutations and PTEN inactivation in GC have been reported to be 4%-25% and 16%-77%, respectively. However, little is known about the association between HER2 expression and PI3K-Akt pathway alterations in GC. Sukawa *et al*[29] have found that HER2 overexpression was significantly correlated with pAkt expression in GC tissues. Furthermore, pAkt expression was correlated with poor prognosis. These results suggest that the PI3K-Akt pathway plays an important role in HER2-positive GC. Moreover, PIK3CA mutations and PTEN inactivation could affect the effectiveness of HER2-targeting therapy. Thus, it is necessary to clarify not only HER2 alterations but also PI3K-Akt pathway alterations to optimize HER2-targeting therapy in patients with GC. In this regard, NGS will be useful for the identification of complicated mechanisms of trastuzumab resistance in GC. The only approved targeted therapy for patients with advanced GC is trastuzumab. It is hoped that NGS will reveal a driver gene alteration that will make other targeted

therapies possible[13,61].

Monoclonal antibodies targeting VEGF (AVAGAST trial) and VEGFR-2 (REGARD trial) in advanced GC

Several vascular endothelial growth factor (VEGF)-targeted agents have been developed, including neutralizing monoclonal antibodies (MoAbs) to VEGF/VEGFRs, soluble VEGF receptors and tyrosine kinase inhibitors (TKIs). The anti-VEGF MoAb bevacizumab has been approved for colorectal cancers. VEGF and VEGF receptor-2 (VEGFR-2)-mediated signaling and angiogenesis contribute to the pathogenesis and progression of GC. The Avastin in Gastric Cancer (AVAGAST) trial was a multinational, randomized, placebo-controlled trial designed to evaluate the efficacy of adding bevacizumab to capecitabine-cisplatin in the first-line treatment of advanced GC[62]. The study showed that adding bevacizumab to the chemotherapy regimen in patients with advanced GC improved the progression-free survival and tumor response rate but not the overall survival. A following biomarker evaluation analysis revealed that plasma VEGF-A and tumor neuropilin-1 are strong biomarker candidates for predicting the clinical outcome in patients with advanced GC treated with bevacizumab[63]. In this regard, NGS will be a powerful method for the identification of predictive biomarkers.

To analyze whether ramucirumab, a monoclonal antibody targeting VEGFR-2, prolongs survival in patients with advanced GC, an international, randomized, double-blind, placebo-controlled, phase 3 trial was conducted in 29 countries[64]. In total, 355 patients with advanced gastric or gastro-esophageal junction adenocarcinoma and disease progression after first-line chemotherapy were randomly assigned (2:1) to receive best supportive care plus either ramucirumab 8 mg/kg (n = 238) or placebo (n = 117), intravenously once every 2 wk. The primary endpoint was overall survival. The median overall survival was 5.2 mo in the ramucirumab group and 3.8 mo in the placebo group (HR = 0.776, 95%CI: 0.603-0.998, P = 0.047). The survival benefit with ramucirumab remained unchanged after multivariate adjustment for other prognostic factors (multivariate HR = 0.774, 95%CI: 0.605-0.991, P = 0.042). Thus, ramucirumab is the first biological treatment given as a single drug that showed survival benefits in patients with advanced gastric or gastro-esophageal junction adenocarcinoma who progressed after first-line chemotherapy. The findings also validate VEGFR-2 signaling as an important therapeutic target in advanced GC.

Potential targeted drugs for GC

Using NGS to target a subset of druggable genes becomes a more effective way to discover therapeutic targets[13,14,61]. There are several potential targeted drugs, either MoAb or small-molecule TKIs, that are being investigated either in synergy with, or in place of, established treatments. These drugs include inhibitors of growth factors and their receptors [*i.e.*, VEGF, epidermal growth factor receptor, HER2, insulin-like growth factor 1 (IGF1) receptor, c-MET], MEK inhibitors and drugs targeting the Hedgehog pathway[65].

Dysregulation of the IGF1 and IGF2/IGF1R system has been implicated in the pathogenesis of GC[66-69]. The expression levels of both IGFs and IGF1R are increased in GC. IGF1R is also involved in angiogenesis and lymphangiogenesis through the modulation of VEGF expression in a GC cell line[70]. IGF1R blockade reduced tumor angiogenesis and enhanced the effects of bevacizumab in a GC cell line. Thus, targeting IGF1R in combination with agents that block the VEGF pathway may have therapeutic utility in GC. Moreover, targeting the novel miR-7/IGF1R/Snail axis has been reported to be useful as a therapeutic approach to block GC metastasis[71].

CONCLUSION

The genetic and epigenetic alterations in GCs continue to inspire biological and clinical implications. Recent advances in the molecular study of GC have brought new diagnostic and therapeutic strategies into clinical settings. The advantages of using DNA methylation as a biomarker for the detection of GC in biopsy specimens and non-invasive body fluids such as serum and gastric washes may have a possible clinical application in GC. Further analysis is required to gain a deeper insight into GC carcinogenesis, a better understanding of disease pathogenesis and the development of new diagnostic and therapeutic approaches targeting essential pathogenic alterations. In this regard, the rapid advances in NGS technologies will hopefully continue to reveal driver alterations of GC, further our understanding of gastric carcinogenesis and improve the therapy for each individual tumor. The characterization of genes that were discovered by NGS rather than by laboratory and clinical research is also necessary.

REFERENCES

1 **Wadhwa R**, Song S, Lee JS, Yao Y, Wei Q, Ajani JA. Gastric cancer-molecular and clinical dimensions. *Nat Rev Clin Oncol* 2013; **10**: 643-655 [PMID: 24061039 DOI: 10.1038/nrclinonc.2013.170]

2 **Akhavan-Niaki H**, Samadani AA. Molecular Insight in Gastric Cancer Induction: An Overview of Cancer Stemness Genes. *Cell Biochem Biophys* 2013 Sep 28; Epub ahead of print [PMID: 24078401]

3 **Figueiredo C**, Garcia-Gonzalez MA, Machado JC. Molecular pathogenesis of gastric cancer. *Helicobacter* 2013; **18** Suppl 1: 28-33 [PMID: 24011242 DOI: 10.1111/hel.12083]

4 **Conteduca V**, Sansonno D, Lauletta G, Russi S, Ingravallo G, Dammacco F. H. pylori infection and gastric cancer: state of the art (review). *Int J Oncol* 2013; **42**: 5-18 [PMID: 23165522 DOI: 10.3892/ijo.2012.1701]

5 **Yamamoto E**, Suzuki H, Takamaru H, Yamamoto H, Toyota M, Shinomura Y. Role of DNA methylation in the development of diffuse-type gastric cancer. *Digestion* 2011; **83**: 241-249 [PMID: 21273772 DOI: 10.1159/000320453]

6 **Baker AM**, Graham TA, Wright NA. Pre-tumour clones, periodic selection and clonal interference in the origin and progression of gastrointestinal cancer: potential for biomarker development. *J Pathol* 2013; **229**: 502-514 [PMID: 23288692 DOI: 10.1002/path.4157]

7 **Meyerson M**, Gabriel S, Getz G. Advances in understanding

cancer genomes through second-generation sequencing. *Nat Rev Genet* 2010; **11**: 685-696 [PMID: 20847746 DOI: 10.1038/nrg2841]
8 **Mardis ER**. A decade's perspective on DNA sequencing technology. *Nature* 2011; **470**: 198-203 [PMID: 21307932 DOI: 10.1038/nature09796]
9 **Patel LR**, Nykter M, Chen K, Zhang W. Cancer genome sequencing: understanding malignancy as a disease of the genome, its conformation, and its evolution. *Cancer Lett* 2013; **340**: 152-160 [PMID: 23111104 DOI: 10.1016/j.canlet.2012.10.018]
10 **Lawrence MS**, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, Meyerson M, Gabriel SB, Lander ES, Getz G. Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature* 2014; **505**: 495-501 [PMID: 24390350 DOI: 10.1038/nature12912]
11 **Lee EJ**, Luo J, Wilson JM, Shi H. Analyzing the cancer methylome through targeted bisulfite sequencing. *Cancer Lett* 2013; **340**: 171-178 [PMID: 23200671 DOI: 10.1016/j.canlet.2012.10.040]
12 **Martens-Uzunova ES**, Olvedy M, Jenster G. Beyond microRNA--novel RNAs derived from small non-coding RNA and their implication in cancer. *Cancer Lett* 2013; **340**: 201-211 [PMID: 23376637 DOI: 10.1016/j.canlet.2012.11.058]
13 **Xuan J**, Yu Y, Qing T, Guo L, Shi L. Next-generation sequencing in the clinic: promises and challenges. *Cancer Lett* 2013; **340**: 284-295 [PMID: 23174106 DOI: 10.1016/j.canlet.2012.11.025]
14 **Ulahannan D**, Kovac MB, Mulholland PJ, Cazier JB, Tomlinson I. Technical and implementation issues in using next-generation sequencing of cancers in clinical practice. *Br J Cancer* 2013; **109**: 827-835 [PMID: 23887607 DOI: 10.1038/bjc.2013.416]
15 **Gerlinger M**, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, Martinez P, Matthews N, Stewart A, Tarpey P, Varela I, Phillimore B, Begum S, McDonald NQ, Butler A, Jones D, Raine K, Latimer C, Santos CR, Nohadani M, Eklund AC, Spencer-Dene B, Clark G, Pickering L, Stamp G, Gore M, Szallasi Z, Downward J, Futreal PA, Swanton C. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med* 2012; **366**: 883-892 [PMID: 22397650 DOI: 10.1056/NEJMoa1113205]
16 **Meacham CE**, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. *Nature* 2013; **501**: 328-337 [PMID: 24048065 DOI: 10.1038/nature12624]
17 **Horswell S**, Matthews N, Swanton C. Cancer heterogeneity and "the struggle for existence": diagnostic and analytical challenges. *Cancer Lett* 2013; **340**: 220-226 [PMID: 23142290 DOI: 10.1016/j.canlet.2012.10.031]
18 **Liang H**, Kim YH. Identifying molecular drivers of gastric cancer through next-generation sequencing. *Cancer Lett* 2013; **340**: 241-246 [PMID: 23178814 DOI: 10.1016/j.canlet.2012.11.029]
19 **Yamamoto H**, Imai K, Perucho M. Gastrointestinal cancer of the microsatellite mutator phenotype pathway. *J Gastroenterol* 2002; **37**: 153-163 [PMID: 11931527]
20 **Perucho M**. Tumors with microsatellite instability: many mutations, targets and paradoxes. *Oncogene* 2003; **22**: 2223-2225 [PMID: 12700658 DOI: 10.1038/sj.onc.1206580]
21 **Imai K**, Yamamoto H. Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. *Carcinogenesis* 2008; **29**: 673-680 [PMID: 17942460 DOI: 10.1093/carcin/bgm228]
22 **Yamamoto H**, Adachi Y, Taniguchi H, Kunimoto H, Nosho K, Suzuki H, Shinomura Y. Interrelationship between microsatellite instability and microRNA in gastrointestinal cancer. *World J Gastroenterol* 2012; **18**: 2745-2755 [PMID: 22719182 DOI: 10.3748/wjg.v18.i22.2745]
23 **Ropero S**, Fraga MF, Ballestar E, Hamelin R, Yamamoto H, Boix-Chornet M, Caballero R, Alaminos M, Setien F, Paz MF, Herranz M, Palacios J, Arango D, Orntoft TF, Aaltonen LA, Schwartz S, Esteller M. A truncating mutation of HDAC2 in human cancers confers resistance to histone deacetylase inhibition. *Nat Genet* 2006; **38**: 566-569 [PMID: 16642021 DOI: 10.1038/ng1773]
24 **Melo SA**, Ropero S, Moutinho C, Aaltonen LA, Yamamoto H, Calin GA, Rossi S, Fernandez AF, Carneiro F, Oliveira C, Ferreira B, Liu CG, Villanueva A, Capella G, Schwartz S, Shiekhattar R, Esteller M. A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function. *Nat Genet* 2009; **41**: 365-370 [PMID: 19219043 DOI: 10.1038/ng.317]
25 **Melo SA**, Moutinho C, Ropero S, Calin GA, Rossi S, Spizzo R, Fernandez AF, Davalos V, Villanueva A, Montoya G, Yamamoto H, Schwartz S, Esteller M. A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells. *Cancer Cell* 2010; **18**: 303-315 [PMID: 20951941 DOI: 10.1016/j.ccr.2010.09.007]
26 **Kim TM**, Laird PW, Park PJ. The landscape of microsatellite instability in colorectal and endometrial cancer genomes. *Cell* 2013; **155**: 858-868 [PMID: 24209623 DOI: 10.1016/j.cell.2013.10.015]
27 **Wang K**, Kan J, Yuen ST, Shi ST, Chu KM, Law S, Chan TL, Kan Z, Chan AS, Tsui WY, Lee SP, Ho SL, Chan AK, Cheng GH, Roberts PC, Rejto PA, Gibson NW, Pocalyko DJ, Mao M, Xu J, Leung SY. Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer. *Nat Genet* 2011; **43**: 1219-1223 [PMID: 22037554 DOI: 10.1038/ng.982]
28 **Zang ZJ**, Cutcutache I, Poon SL, Zhang SL, McPherson JR, Tao J, Rajasegaran V, Heng HL, Deng N, Gan A, Lim KH, Ong CK, Huang D, Chin SY, Tan IB, Ng CC, Yu W, Wu Y, Lee M, Wu J, Poh D, Wan WK, Rha SY, So J, Salto-Tellez M, Yeoh KG, Wong WK, Zhu YJ, Futreal PA, Pang B, Ruan Y, Hillmer AM, Bertrand D, Nagarajan N, Rozen S, Teh BT, Tan P. Exome sequencing of gastric adenocarcinoma identifies recurrent somatic mutations in cell adhesion and chromatin remodeling genes. *Nat Genet* 2012; **44**: 570-574 [PMID: 22484628 DOI: 10.1038/ng.2246]
29 **Sukawa Y**, Yamamoto H, Nosho K, Kunimoto H, Suzuki H, Adachi Y, Nakazawa M, Nobuoka T, Kawayama M, Mikami M, Matsuno T, Hasegawa T, Hirata K, Imai K, Shinomura Y. Alterations in the human epidermal growth factor receptor 2-phosphatidylinositol 3-kinase-v-Akt pathway in gastric cancer. *World J Gastroenterol* 2012; **18**: 6577-6586 [PMID: 23236232 DOI: 10.3748/wjg.v18.i45.6577]
30 **Sukawa Y**, Yamamoto H, Nosho K, Ito M, Igarashi H, Naito T, Mitsuhashi K, Matsunaga Y, Takahashi T, Mikami M, Adachi Y, Suzuki H, Shinomura Y. HER2 expression and PI3K-Akt pathway alterations in gastric cancer. *Digestion* 2014; **89**: 12-17 [PMID: 24458107 DOI: 10.1159/000356201]
31 **Holbrook JD**, Parker JS, Gallagher KT, Halsey WS, Hughes AM, Weigman VJ, Lebowitz PF, Kumar R. Deep sequencing of gastric carcinoma reveals somatic mutations relevant to personalized medicine. *J Transl Med* 2011; **9**: 119 [PMID: 21781349 DOI: 10.1186/1479-5876-9-119]
32 **Lei Z**, Tan IB, Das K, Deng N, Zouridis H, Pattison S, Chua C, Feng Z, Guan YK, Ooi CH, Ivanova T, Zhang S, Lee M, Wu J, Ngo A, Manesh S, Tan E, Teh BT, So JB, Goh LK, Boussioutas A, Lim TK, Flotow H, Tan P, Rozen SG. Identification of molecular subtypes of gastric cancer with different responses to PI3-kinase inhibitors and 5-fluorouracil. *Gastroenterology* 2013; **145**: 554-565 [PMID: 23684942 DOI: 10.1053/j.gastro.2013.05.010]
33 **Abe H**, Maeda D, Hino R, Otake Y, Isogai M, Ushiku AS, Matsusaka K, Kunita A, Ushiku T, Uozaki H, Tateishi Y, Hishima T, Iwasaki Y, Ishikawa S, Fukayama M. ARID1A expression loss in gastric cancer: pathway-dependent roles with and without Epstein-Barr virus infection and microsatellite instability. *Virchows Arch* 2012; **461**: 367-377 [PMID:

22915242 DOI: 10.1007/s00428-012-1303-2]
34 **Nagarajan N**, Bertrand D, Hillmer AM, Zang ZJ, Yao F, Jacques PE, Teo AS, Cutcutache I, Zhang Z, Lee WH, Sia YY, Gao S, Ariyaratne PN, Ho A, Woo XY, Veeravali L, Ong CK, Deng N, Desai KV, Khor CC, Hibberd ML, Shahab A, Rao J, Wu M, Teh M, Zhu F, Chin SY, Pang B, So JB, Bourque G, Soong R, Sung WK, Tean Teh B, Rozen S, Ruan X, Yeoh KG, Tan PB, Ruan Y. Whole-genome reconstruction and mutational signatures in gastric cancer. *Genome Biol* 2012; **13**: R115 [PMID: 23237666 DOI: 10.1186/gb-2012-13-12-r115]
35 **Deng N**, Goh LK, Wang H, Das K, Tao J, Tan IB, Zhang S, Lee M, Wu J, Lim KH, Lei Z, Goh G, Lim QY, Tan AL, Sin Poh DY, Riahi S, Bell S, Shi MM, Linnartz R, Zhu F, Yeoh KG, Toh HC, Yong WP, Cheong HC, Rha SY, Boussioutas A, Grabsch H, Rozen S, Tan P. A comprehensive survey of genomic alterations in gastric cancer reveals systematic patterns of molecular exclusivity and co-occurrence among distinct therapeutic targets. *Gut* 2012; **61**: 673-684 [PMID: 22315472 DOI: 10.1136/gutjnl-2011-301839]
36 **Melo SA**, Esteller M. Dysregulation of microRNAs in cancer: playing with fire. *FEBS Lett* 2011; **585**: 2087-2099 [PMID: 20708002 DOI: 10.1016/j.febslet.2010.08.009]
37 **Cortez MA**, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids--the mix of hormones and biomarkers. *Nat Rev Clin Oncol* 2011; **8**: 467-477 [PMID: 21647195 DOI: 10.1038/nrclinonc.2011.76]
38 **van Kouwenhove M**, Kedde M, Agami R. MicroRNA regulation by RNA-binding proteins and its implications for cancer. *Nat Rev Cancer* 2011; **11**: 644-656 [PMID: 21822212 DOI: 10.1038/nrc3107]
39 **Lopez-Serra P**, Esteller M. DNA methylation-associated silencing of tumor-suppressor microRNAs in cancer. *Oncogene* 2012; **31**: 1609-1622 [PMID: 21860412 DOI: 10.1038/onc.2011.354]
40 **Mitchell PS**, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008; **105**: 10513-10518 [PMID: 18663219 DOI: 10.1073/pnas.0804549105]
41 **Ueda T**, Volinia S, Okumura H, Shimizu M, Taccioli C, Rossi S, Alder H, Liu CG, Oue N, Yasui W, Yoshida K, Sasaki H, Nomura S, Seto Y, Kaminishi M, Calin GA, Croce CM. Relation between microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis. *Lancet Oncol* 2010; **11**: 136-146 [PMID: 20022810 DOI: 10.1016/S1470-2045(09)70343-2]
42 **Tong F**, Cao P, Yin Y, Xia S, Lai R, Liu S. MicroRNAs in gastric cancer: from benchtop to bedside. *Dig Dis Sci* 2014; **59**: 24-30 [PMID: 24114043]
43 **Pan HW**, Li SC, Tsai KW. MicroRNA dysregulation in gastric cancer. *Curr Pharm Des* 2013; **19**: 1273-1284 [PMID: 23092346 DOI: 10.2174/138161213804805621]
44 **Albulescu R**, Neagu M, Albulescu L, Tanase C. Tissular and soluble miRNAs for diagnostic and therapy improvement in digestive tract cancers. *Expert Rev Mol Diagn* 2011; **11**: 101-120 [PMID: 21171925 DOI: 10.1586/erm.10.106]
45 **Li SC**, Liao YL, Ho MR, Tsai KW, Lai CH, Lin WC. miRNA arm selection and isomiR distribution in gastric cancer. *BMC Genomics* 2012; **13 Suppl 1**: S13 [PMID: 22369582 DOI: 10.1186/1471-2164-13-S1-S13]
46 **Kim YH**, Liang H, Liu X, Lee JS, Cho JY, Cheong JH, Kim H, Li M, Downey TJ, Dyer MD, Sun Y, Sun J, Beasley EM, Chung HC, Noh SH, Weinstein JN, Liu CG, Powis G. AMPKα modulation in cancer progression: multilayer integrative analysis of the whole transcriptome in Asian gastric cancer. *Cancer Res* 2012; **72**: 2512-2521 [PMID: 22434430 DOI: 10.1158/0008-5472.CAN-11-3870]
47 **Azad N**, Zahnow CA, Rudin CM, Baylin SB. The future of epigenetic therapy in solid tumours--lessons from the past. *Nat Rev Clin Oncol* 2013; **10**: 256-266 [PMID: 23546521 DOI: 10.1038/nrclinonc.2013.42]
48 **Zouridis H**, Deng N, Ivanova T, Zhu Y, Wong B, Huang D, Wu YH, Wu Y, Tan IB, Liem N, Gopalakrishnan V, Luo Q, Wu J, Lee M, Yong WP, Goh LK, Teh BT, Rozen S, Tan P. Methylation subtypes and large-scale epigenetic alterations in gastric cancer. *Sci Transl Med* 2012; **4**: 156ra140 [PMID: 23076357 DOI: 10.1126/scitranslmed.3004504]
49 **Gigek CO**, Chen ES, Calcagno DQ, Wisnieski F, Burbano RR, Smith MA. Epigenetic mechanisms in gastric cancer. *Epigenomics* 2012; **4**: 279-294 [PMID: 22690664 DOI: 10.2217/epi.12.22]
50 **Qu Y**, Dang S, Hou P. Gene methylation in gastric cancer. *Clin Chim Acta* 2013; **424**: 53-65 [PMID: 23669186 DOI: 10.1016/j.cca.2013.05.002]
51 **Calcagno DQ**, Gigek CO, Chen ES, Burbano RR, Smith Mde A. DNA and histone methylation in gastric carcinogenesis. *World J Gastroenterol* 2013; **19**: 1182-1192 [PMID: 23482412 DOI: 10.3748/wjg.v19.i8.1182]
52 **Otani K**, Li X, Arakawa T, Chan FK, Yu J. Epigenetic-mediated tumor suppressor genes as diagnostic or prognostic biomarkers in gastric cancer. *Expert Rev Mol Diagn* 2013; **13**: 445-455 [PMID: 23782252 DOI: 10.1586/erm.13.32]
53 **Takamaru H**, Yamamoto E, Suzuki H, Nojima M, Maruyama R, Yamano HO, Yoshikawa K, Kimura T, Harada T, Ashida M, Suzuki R, Yamamoto H, Kai M, Tokino T, Sugai T, Imai K, Toyota M, Shinomura Y. Aberrant methylation of RASGRF1 is associated with an epigenetic field defect and increased risk of gastric cancer. *Cancer Prev Res* (Phila) 2012; **5**: 1203-1212 [PMID: 22961779 DOI: 10.1158/1940-6207.CAPR-12-0056]
54 **Suzuki H**, Yamamoto E, Nojima M, Kai M, Yamano HO, Yoshikawa K, Kimura T, Kudo T, Harada E, Sugai T, Takamaru H, Niinuma T, Maruyama R, Yamamoto H, Tokino T, Imai K, Toyota M, Shinomura Y. Methylation-associated silencing of microRNA-34b/c in gastric cancer and its involvement in an epigenetic field defect. *Carcinogenesis* 2010; **31**: 2066-2073 [PMID: 20924086 DOI: 10.1093/carcin/bgq203]
55 **Suzuki R**, Yamamoto E, Nojima M, Maruyama R, Yamano HO, Yoshikawa K, Kimura T, Harada T, Ashida M, Niinuma T, Sato A, Nosho K, Yamamoto H, Kai M, Sugai T, Imai K, Suzuki H, Shinomura Y. Aberrant methylation of microRNA-34b/c is a predictive marker of metachronous gastric cancer risk. *J Gastroenterol* 2013 Aug 13; Epub ahead of print [PMID: 23942619]
56 **Watanabe Y**, Kim HS, Castoro RJ, Chung W, Estecio MR, Kondo K, Guo Y, Ahmed SS, Toyota M, Itoh F, Suk KT, Cho MY, Shen L, Jelinek J, Issa JP. Sensitive and specific detection of early gastric cancer with DNA methylation analysis of gastric washes. *Gastroenterology* 2009; **136**: 2149-2158 [PMID: 19375421 DOI: 10.1053/j.gastro.2009.02.085]
57 **Oishi Y**, Watanabe Y, Yoshida Y, Sato Y, Hiraishi T, Oikawa R, Maehata T, Suzuki H, Toyota M, Niwa H, Suzuki M, Itoh F. Hypermethylation of Sox17 gene is useful as a molecular diagnostic application in early gastric cancer. *Tumour Biol* 2012; **33**: 383-393 [PMID: 22161215 DOI: 10.1007/s13277-011-0278-y]
58 **Baba S**, Oishi Y, Watanabe Y, Oikawa R, Morita R, Yoshida Y, Hiraishi T, Maehata T, Nagase Y, Fukuda Y, Nakazawa M, Ishigouoka S, Hattori N, Suzuki H, Toyota M, Niwa H, Suzuki M, Itoh F. Gastric wash-based molecular testing for antibiotic resistance in Helicobacter pylori. *Digestion* 2011; **84**: 299-305 [PMID: 22057261 DOI: 10.1159/000332570]
59 **Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or

gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210 DOI: 10.1016/S0140-6736(10)61121-X]

60 **de Mello RA**, Marques AM, Araújo A. HER2 therapies and gastric cancer: a step forward. *World J Gastroenterol* 2013; **19**: 6165-6169 [PMID: 24115812]

61 **Meric-Bernstam F**, Mills GB. Overcoming implementation challenges of personalized cancer therapy. *Nat Rev Clin Oncol* 2012; **9**: 542-548 [PMID: 22850751 DOI: 10.1038/nrclinonc.2012.127]

62 **Ohtsu A**, Shah MA, Van Cutsem E, Rha SY, Sawaki A, Park SR, Lim HY, Yamada Y, Wu J, Langer B, Starnawski M, Kang YK. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. *J Clin Oncol* 2011; **29**: 3968-3976 [PMID: 21844504 DOI: 10.1200/JCO.2011.36.2236]

63 **Van Cutsem E**, de Haas S, Kang YK, Ohtsu A, Tebbutt NC, Ming Xu J, Peng Yong W, Langer B, Delmar P, Scherer SJ, Shah MA. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a biomarker evaluation from the AVAGAST randomized phase III trial. *J Clin Oncol* 2012; **30**: 2119-2127 [PMID: 22565005 DOI: 10.1200/JCO.2011.39.9824]

64 **Fuchs CS**, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, Safran H, dos Santos LV, Aprile G, Ferry DR, Melichar B, Tehfe M, Topuzov E, Zalcberg JR, Chau I, Campbell W, Sivanandan C, Pikiel J, Koshiji M, Hsu Y, Liepa AM, Gao L, Schwartz JD, Tabernero J. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 2014; **383**: 31-39 [PMID: 24094768 DOI: 10.1016/S0140-6736(13)61719-5]

65 **Matsubara J**, Yamada Y, Hirashima Y, Takahari D, Okita NT, Kato K, Hamaguchi T, Shirao K, Shimada Y, Shimoda T. Impact of insulin-like growth factor type 1 receptor, epidermal growth factor receptor, and HER2 expressions on outcomes of patients with gastric cancer. *Clin Cancer Res* 2008; **14**: 3022-3029 [PMID: 18483367 DOI: 10.1158/1078-0432.CCR-07-1898]

66 **Adachi Y**, Li R, Yamamoto H, Min Y, Piao W, Wang Y, Imsumran A, Li H, Arimura Y, Lee CT, Imai K, Carbone DP, Shinomura Y. Insulin-like growth factor-I receptor blockade reduces the invasiveness of gastrointestinal cancers via blocking production of matrilysin. *Carcinogenesis* 2009; **30**: 1305-1313 [PMID: 19493905 DOI: 10.1093/carcin/bgp134]

67 **Adachi Y**, Yamamoto H, Ohashi H, Endo T, Carbone DP, Imai K, Shinomura Y. A candidate targeting molecule of insulin-like growth factor-I receptor for gastrointestinal cancers. *World J Gastroenterol* 2010; **16**: 5779-5789 [PMID: 21154998 DOI: 10.3748/wjg.v16.i46.5779]

68 **Popa EC**, Shah MA. Met, IGF1R, and other new targets in upper GI malignancies. *Curr Treat Options Oncol* 2013; **14**: 321-336 [PMID: 23873272 DOI: 10.1007/s11864-013-0245-5]

69 **Singh P**, Alex JM, Bast F. Insulin receptor (IR) and insulin-like growth factor receptor 1 (IGF-1R) signaling systems: novel treatment strategies for cancer. *Med Oncol* 2014; **31**: 805 [PMID: 24338270 DOI: 10.1007/s12032-013-0805-3]

70 **Li H**, Adachi Y, Yamamoto H, Min Y, Ohashi H, Ii M, Arimura Y, Endo T, Lee CT, Carbone DP, Imai K, Shinomura Y. Insulin-like growth factor-I receptor blockade reduces tumor angiogenesis and enhances the effects of bevacizumab for a human gastric cancer cell line, MKN45. *Cancer* 2011; **117**: 3135-3147 [PMID: 21264842 DOI: 10.1002/cncr.25893]

71 **Zhao X**, Dou W, He L, Liang S, Tie J, Liu C, Li T, Lu Y, Mo P, Shi Y, Wu K, Nie Y, Fan D. MicroRNA-7 functions as an anti-metastatic microRNA in gastric cancer by targeting insulin-like growth factor-1 receptor. *Oncogene* 2013; **32**: 1363-1372 [PMID: 22614005 DOI: 10.1038/onc.2012.156]

P- Reviewers: Deng SL, Reim D, Sperti C **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Ma S

百世登
Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

ISSN 1007-9327
9 771007 932045 14>

Int J Hematol (2014) 100:296–306
DOI 10.1007/s12185-014-1632-9

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

Kazuteru Ohashi · Tokiko Nagamura-Inoue · Fumitaka Nagamura · Arinobu Tojo · Kouichi Miyamura · Takehiko Mori · Mineo Kurokawa · Shuichi Taniguchi · Jun Ishikawa · Yasuo Morishima · Yoshiko Atsuta · Hisashi Sakamaki

Received: 19 October 2013 / Revised: 1 July 2014 / Accepted: 2 July 2014 / Published online: 2 August 2014

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.

Electronic supplementary material The online version of this article (doi:10.1007/s12185-014-1632-9) contains supplementary material, which is available to authorized users.

K. Ohashi (✉) · H. Sakamaki
Hematology Division, Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital, Tokyo, Japan
e-mail: k.ohashi@cick.jp

T. Nagamura-Inoue
Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

T. Nagamura-Inoue
Japan Cord Blood Bank Network, Tokyo, Japan

F. Nagamura
Division of Clinical Trial Safety Management, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

A. Tojo
Department of Hematology/Oncology, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

K. Miyamura
Department of Hematology, Japanese Red Cross Nagoya Daiichi Hospital, Nagoya, Japan

T. Mori
Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan

M. Kurokawa
Department of Cell Therapy and Transplantation Medicine, The University of Tokyo, Tokyo, Japan

S. Taniguchi
Department of Hematology, Toranomon Hospital, Tokyo, Japan

J. Ishikawa
Department of Hematology and Chemotherapy, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan

Y. Morishima
Division of Epidemiology/Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan

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

Y. Morishima
Japan Marrow Donor Program, Tokyo, Japan

Y. Atsuta
Department of HSCT Data Management/Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan

Y. Atsuta · H. Sakamaki
Japanese Society of Hematopoietic Cell Transplantation, Nagoya, Japan

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	338/193 ($P < 0.001$)	215/127 ($P < 0.001$)	123/66 ($P < 0.001$)
Male/female			
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	133/249[b]	187/108[b]	94/95 ($P = 0.94$)
Yes/no	($P < 0.001$)	($P < 0.001$)	
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

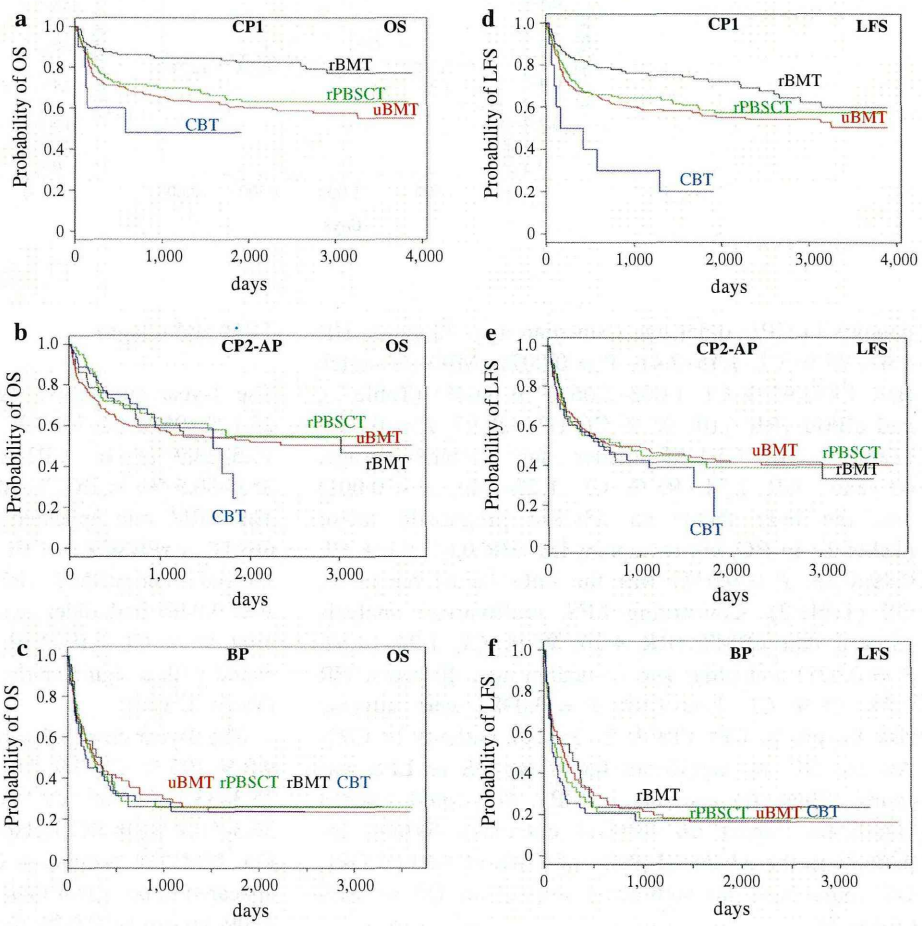

Fig. 1 Kaplan–Meier estimate of overall survival (OS) for patients in CP1 (**a**), CP2-AP (**b**) and BC (**c**); and leukemia-free survival (LFS) for patients in CP1 (**d**), CP2-AP (**e**) and BC (**f**)