Principles of haematopoietic transplantation

The terms ‘haematopoietic stem cell’ and ‘stem cell transplantation’ have been defined by Little and Storb (2002): “A primitive and immature cell of the haematopoietic system that has the capacity to give rise to all the cells of the blood system, as well as the ability to self-renew. Allogeneic haematopoietic stem cell transplantation involves the transfer of both immature and mature blood cells from the bone marrow, peripheral blood or cord blood from one individual to another.” The first experiences in haematopoietic transplantation have been made in mice in the late 1940s and the early 1950s by Jacobson et al. (1949) and Lorenz et al. (1951) in the wake of the first atomic bomb explosions in Japan and its life-threatening effects due to bone marrow failure. Since then, every decade is featured by revolutionary developments. Epigenetic approaches might be the discovery of the 2010s.

Indications for allogeneic haematopoietic transplantation are both malignant and nonmalignant diseases; most of all transplantations are administered for haematological malignancies, e.g. acute and chronic leukaemias, myelodysplasia and myeloproliferative disorders.

The procedure of allogeneic stem cell transplantation can be divided into four main phases as summarized in Table 1. In addition to irradiation and high-dose chemotherapy, residual malignant cells are eliminated by the graft-versus-leukaemia reaction (GvL). The beneficial effect of this immunological mechanism is limited by the most frequent complication of stem cell transplantation: graft-versus-host disease (GvHD).

Pathogenesis of acute GvHD is explained by the three-phase model proposed by Ferrara et al. (2009). At first, tissue damage caused by high-dose chemotherapy and irradiation (conditioning regimen) induces translocation of bacterial products (lipopolysaccharides) through gut mucosa or skin leakage and stimulates proinflammatory cytokine release: interleukin (IL)-1, IL-6 and tumour necrosis factor alpha (TNF-α). Thus, host antigen-presenting cells (APCs) are activated and migrate into secondary lymphoid organs. In the second phase, activated host APCs induce proliferation and cytokine production (IL-2 and interferon gamma, INF-γ) of donor T lymphocytes by presenting alloantigens. Furthermore, donor T cells differentiate into alloreactive effector T cells against different alloantigens (minor histocompatibility antigens). In the last step, two important components mediate inflammation: cellular effectors like activated alloreactive effector T cells and natural killer (NK) cells on the one hand and soluble inflammatory cytokines as TNF-α, IFN-γ and IL-1 on the other hand. This in turn causes target cell apoptosis and thereby enhances alloantigen presentation as well as cytokine release, thus amplifying and sustaining the inflammatory reaction.

Acute GvHD occurring within the first months after transplantation must be differentiated from chronic GvHD, whose pathophysiology still remains unclear. Depending on organ involvement, patients with acute GvHD suffer from skin rash up to blisters and ulcers, severe diarrhoea and wasting syndrome or elevated liver enzymes up to liver failure.

However, alloreactive T cells do not only recognize solid organ tissue like skin, gut and liver, but also residual malignant cells. This favourable effect named graft-versus-leukaemia
reaction has been observed especially in myeloid leukaemias (Kolb 2008) and is the great benefit of allogeneic transplantation. Therefore, the intention is prevention and treatment of GvHD while preserving GvL.

**GvHD prevention and treatment—preservation of GvL**

With regard to epigenetic targets, there are two important cellular mechanisms: regulatory T cells suppress GvHD without altering GvL, and NK cells and killer cell immunoglobulin-like receptors enhance GvL without inducing GvHD.

**Regulatory T cells—maintenance of (self-) tolerance**

Regulatory T cells (Tregs) are known to regulate inflammatory response and suppress alloreactive T cells; the exact mechanism is unknown so far. They suppress autoimmunity and GvHD without decreasing GvL (Edinger et al. 2003). Mice deficient for Tregs usually suffer from autoimmune diseases, and even in humans, autoimmune disorders are often accompanied with Tregs dysfunction, e.g. Forkhead transcription factor Foxp3 gene mutation (Fontenot et al. 2003; Bennett et al. 2001). Recent data suggest that FOXP3 is necessary and essential for sufficient and functional Tregs that are defined as CD4+CD25+FOXP3+ T cells. The Foxp3 locus is regulated by epigenetic modifications like acetylation and methylation (Floess et al. 2007; Tao et al. 2007) and is unmethylated in active Tregs. Regulatory T cells CD4+CD25+FOX3+ are mainly generated in the thymus, but may also arise from naïve CD4+CD25− T cells in the periphery by T cell-receptor stimulation. The number of circulating Tregs in vivo is low, and purification methods in vitro still remain inefficient. The ambitious attempt of in vitro expansion of Tregs also failed so far because of loss of suppressor function of expanded Tregs possibly due to inactive Foxp3 (Oliveira et al. 2008). As reported by Floess et al. (2007) and Tao et al. (2007), FOXP3 expression must be stabilized by epigenetic modifications such as complete demethylation of a highly conserved region within the noncoding region of FOX3 and acetylation of lysine residues in the forkhead domain by inhibition of HDACs.

**Natural killer cells: KIR expression and alloreactivity**

NK cells have been shown to have alloreactive potential in the donor–recipient direction and induce tumour cell lysis without immune sensitization of the recipient before (Colonna et al. 1993; Ciccone et al. 1992; Kiessling et al. 1975). As recently reviewed by Pegram et al. (2011), NK cell activity is regulated by inhibitory and activating killer cell immunoglobulin-like receptors (KIR) whose ligands are major histocompatibility complex (MHC) class I molecules. If MHC class I ligands for inhibitory KIR are missing on target cells, NK cells are activated and mediate cell lysis with preference against tumour cells. That implies reaction against leukaemia (GvL) without GvHD. There is evidence that enhanced KIR mismatch in haploidentical transplantation setting is followed by an intensified immunological reaction and boosts graft versus leukaemia effect (Apperley et al. 2008; Ruggeri et al. 2002; Pende et al. 2005).

**Epigenetic targets in HSCT**

Epigenetic active agents can be divided into two main groups: histone deacetylase (HDAC) inhibitors like vorinostat and panobinostat and DNA methyltransferase (DNMT) inhibitors like 5-azacytidine and decitabine. With respect to promising preclinical data, a lot of translational research still has to be performed to further establish these drugs in clinical routine. When used in the transplantation setting, the potential benefit of HDAC...
and DNMT inhibitors has to be specified corresponding to the main phases of transplantation.

1. Epigenetic agents prior to HSCT

Low disease burden prior to haematopoietic transplantation is known to come along with favourable outcome and reduced relapse incidence, although treatment-related mortality is increased by pretransplant induction chemotherapy. The beneficial antileukaemic effect of epigenetic active drugs by activation of silenced genes, derepression of tumour suppressor genes and induction of differentiation can be used by adding DNMT and HDAC inhibitors to the conditioning regimen or cytoreductive chemotherapy.

DNMT inhibitors: decitabine and 5-azacytidine

Treatment with decitabine or 5-azacytidine before or in combination with common myeloablative or non-myeloablative conditioning regimen seems to be a promising approach with respectable results as shown in several phase I/II studies in mainly pretreated and refractory AML and MDS patients (De Padua Silva et al. 2007; Lubbert et al. 2006; McCarty et al. 2008; de Lima et al. 2003; Giralt et al. 1997; Table 2). Rates of complete remission prior to transplantation vary from 30% up to 90%, and no unexpected side effects have been reported (De Padua Silva et al. 2007; de Lima et al. 2003; Fontenot et al. 2005). Most patients suffer from gastrointestinal toxicity and neutropenic infection (De Padua Silva et al. 2007). Against previous reports about delayed engraftment after application of hypomethylating agents prior to transplantation (Giralt et al. 1997), this observation could not be confirmed in recent studies (De Padua Silva et al. 2007; Lubbert et al. 2006; McCarty et al. 2008; de Lima et al. 2003). In conclusion, additional application of hypomethylating agents prior to transplantation is safe and might be a favourable option for disease control to bridge time period to transplant.

HDAC inhibitors

There are no clinical data available for the use of HDAC inhibitors prior to haematopoietic transplantation so far. Wang et al. report reduction of myelofibrosis in mice with JAK2+ primary myelofibrosis (PMF) when PMF cells are treated sequentially with decitabine and SAHA (vorinostat) or trichostatin A in vitro prior to transplantation (Wang et al. 2010). Further investigations are necessary to estimate a potential benefit of HDAC inhibitors prior to stem cell transplantation.

2. Epigenetic agents as immunomodulatory therapy

As mentioned above, the clinical goal of stem cell transplantation is reduction of GvHD while preserving GvL. Key mechanisms in the treatment of GvHD without altering GvL effect are regulation of cytokine levels, the interfering function of regulatory T cells (Tregs) and the important role of natural killer (NK) cells.

Promising preclinical data demonstrate the potent immunomodulatory effect of both HDAC and DNMT inhibitors in the treatment of GvHD without reducing the beneficial effect of GvL.

Regulation of cytokine level

Proinflammatory cytokines like TNF-α, IFN-γ, IL-1, IL-6 and IL-12 are essential mediators of GvHD sustaining the vicious circle of inflammation. HDAC inhibitor vorinostat (SAHA) has been shown to inhibit the production of proinflammatory cytokines TNF-α, IFN-γ, IL-1β and IL-12 in lipopolysaccharide-stimulated human peripheral

| Author     | n | Agent | Disease          | Remission | Outcome after HSCT | [median] Follow-up (months) | Reference              |
|------------|---|-------|------------------|-----------|--------------------|----------------------------|-------------------------|
| Lübbert    | 10| Dec   | AML/MDS         | 40% CR, 10% PR | 33% relapse, 33% alive | 26/10/1                   | (Lubbert et al. 2006)   |
| De Padua   | 12| Dec   | MDS             | 33% CR, 50% PR | 17% relapse, 75% alive | 11.5                      | (De Padua Silva et al. 2007) |
| McCarty    | 25| 5-Aza | AML/MDS         | 52% ORR    |                    | 12                        | (McCarty et al. 2008)   |
| de Lima    | 23| Dec   | 12× AML, 1×CMML, 1× ALL, 9× CML | 91% CR | 39% relapse, 26% alive | 39                       | (de Lima et al. 2003)   |
| Giralt     | 4 | Dec   | 3× CML, 1× AMML | 2× CR     | 3× alive           | 6                         | (Giralt et al. 1997)    |

Dec decitabine, 5-Aza 5-azacytidine, AML acute myeloid leukaemia, MDS myelodysplasia, CMML chronic myelomonocytic leukaemia, CML chronic myeloid leukaemia, ALL acute lymphoblastic leukaemia, AMML acute myelomonocytic leukaemia, CR complete remission, PR partial remission, ORR overall response rate
Addition of SAHA day +3 to +7 after transplantation prevents gastrointestinal tract damage by reducing cytokine release of TNF-α, IFN-γ and IL-1 in a dose-dependent manner. When compared with allogeneic controls, mortality and grade of acute GvHD were reduced corresponding to significantly improved survival (Reddy et al. 2004). Surprisingly, prophylactic treatment with SAHA did not alter cytotoxic T cell reaction against host antigens and thereby preserved GvL effect.

Epigenetic agents boost regulatory T cells (Tregs)

CD4+CD25+FOXP3+ Tregs are suppressors of autoimmunity and GvHD but do not reduce GvL effect; the exact mechanism is still not known. The circulating number of functional Tregs in vivo is limited and effective in vitro expansion, and purification methods are not available so far. DNMT inhibitors decitabine and 5-azacytidine as well as HDAC inhibitors have been reported to be potential stimulators of Tregs by inducing Foxp3 expression in CD4+CD25+FOXP3− T cells. Foxp3 is regulated by methylation and acetylation and is highly hypermethylated in nonfunctional Tregs. Treatment of mice with decitabine and 5-azacytidine after bone marrow transplantation expands Tregs, enhances the circulating number of functional Tregs by expression of Foxp3 and thereby limits GvHD without sacrificing GvL (Sanchez-Abarca et al. 2010; Choi et al. 2010). Application of HDAC inhibitors (trichostatin A, valproic acid and butyrate) in mice provides similar results (Tao et al. 2007).

DNMT inhibitors and natural killer cells

In clinical trials, treatment with DNMT inhibitors after haematopoietic stem cell transplantation (HSCT) prevents relapse and even induces durable remission in relapsed situation probably by enhancing GvL effect (Giralt et al. 1997; Czibere et al. 2006; Ravandi et al. 2001; Jabbour et al. 2009; de Lima et al. 2007). NK cells play a key role in the pathophysiology of graft-versus-leukaemia reaction by inducing cell lysis with priority against tumour cells. In the haploidentical transplantation setting, GvL effect is enhanced by KIR mismatch as mentioned above. Chan et al. (2003) demonstrated that KIR expression in NK cells is regulated by methylation: KIR hypomethylation correlates with KIR expression. Moreover, treatment of NK cells with decitabine as methyltransferase (DNMT) inhibitor induces KIR expression and thereby enhances KIR variability. These facts suggest that treatment with DNMT inhibitors like decitabine might induce GvL effect due to tumour cell lysis by NK cells by enhanced KIR expression and variability.

3. DNMT inhibitors as relapse therapy and maintenance after HSCT

Patients with leukaemic relapse after stem cell transplantation have a very poor prognosis, and treatment options are limited because of accumulated toxicity and impaired organ function. DNMT inhibitors decitabine and 5-azacytidine have been shown to be effective antileukaemic agents with acceptable toxicity profile that can be used safely in relapsed situation after HSCT (Giralt et al. 1997; Ravandi et al. 2001; Jabbour et al. 2009) (Table 3).

| Author       | n  | Agent   | Disease     | Duration            | Outcome      | Ref.                          |
|--------------|----|---------|-------------|---------------------|--------------|------------------------------|
| Giralt 1997  | 3  | Dec     | 2× AML, 1× ALL | 3× CR              | 1× relapse, 1× alive | (Giralt et al. 1997)         |
| Ravandi 2001 | 14 | Dec+HSCT| 9× AML, 2× ALL, 3× CML | 8× CR/PR         | 5× relapse, 5× alive | (Ravandi et al. 2001)        |
| Jabbour 2009 | 9  | 5-Aza   | AML         | 5× CR/PR           | 1× relapse, 7× alive | (Jabbour et al. 2009)        |
| Czibere 2006 | 6  | 5-Aza+DLI | AML/MDS  | 3× CR, 2× PR       | 2× relapse, 2× alive | (Czibere et al. 2006)        |

Table 4 5-Azacytidine (5-Aza) as maintenance therapy after haematopoietic transplantation (HSCT)

| Author       | n  | Agent  | Disease    | Duration                  | Outcome               | Ref.                        |
|--------------|----|--------|------------|---------------------------|-----------------------|-----------------------------|
| de Lima 2007 | 40 | 5-Aza  | AML/MDS    | up to 4 cycles at 28 days | 11× relapse           | (de Lima et al. 2007)       |
| Jabbour 2009 | 8  | 5-Aza (+3× HSCT) | 7× AML, 1× ALL | median 8 cycles à 28 days (up to 22 cycles) | 3× relapse, 7× alive   | (Jabbour et al. 2009)       |

AML acute myeloid leukaemia, MDS myelodysplasia, ALL acute lymphoblastic leukaemia
Treatment with 5-azacytidine might even enhance GvL effect especially in combination with donor lymphocyte infusions as reported by Czibere et al. (2006). Furthermore, maintenance therapy with 5-azacytidine after stem cell transplantation might induce durable remission without increasing acute GvHD (Jabbour et al. 2009; de Lima et al. 2007; Table 4).

Conclusion

Recent clinical and preclinical data suggest the use of epigenetic active drugs as a promising new approach in stem cell transplantation in the 2010s. DNMT and HDAC inhibitors show high antitumour activity when both used as additional agents in conditioning regimen and as maintenance therapy or even for remission induction in relapsed situation after transplantation. When used in heavily pretreated patients, the favourable toxicity profile indicates safety and limited short-term side effects. Long-term side effects are not known so far. With respect to their impact on expression pattern of a wide range of genes and functionality of proteins, DNMT and HDAC inhibitors might interfere with different biological mechanisms. Induction of immunotolerance and anti-inflammation might even cause higher incidence of malignancies after long-term treatment. Furthermore, reduction of immune response might be supposed to result in an increased risk for opportunistic and other infections. Further studies are mandatory to evaluate the potential and safety of epigenetic agents in a higher number of cases.

Preclinical data indicate a beneficial immunomodulatory effect of DNMT and HDAC inhibitors by enhancing functional Tregs, regulating inflammatory cytokines and inducing GvL effect by enhancing KIR expression and variability of NK cells. Clinical data are still missing so far, and clinical studies should be investigated to verify the use of HDAC and DNMT inhibitors for GvHD prophylaxis and therapy.

Conflict of interest The authors declare that they have no conflict of interest.

References

Apperley J et al (2008) The EBMT handbook: haematopoietic stem cell transplantation. European Sch of Haematology 5:66–75
Bennett CL et al (2001) The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 27(1):20–21
Chan HW et al (2003) DNA methylation maintains allele-specific KIR gene expression in human natural killer cells. J Exp Med 197(2):245–255
Choi J et al (2010) In vivo administration of hypomethylating agents mitigate graft-versus-host disease without sacrificing graft-versus-leukemia. Blood 116(1):129–139
Ciccone E et al (1992) Evidence of a natural killer (NK) cell repertoire for (allo) antigen recognition: definition of five distinct NK-determined allospecificities in humans. J Exp Med 175(3):709–718
Colonna M et al (1993) Generation of allospecific natural killer cells by stimulation across a polymorphism of HLA-C. Science 260(5111):1121–1124
Czibere A et al (2006) 5-Azacytidine in combination with donor lymphocyte infusions for the treatment of patients with MDS or AML relapsing after allogeneic stem cell transplantation. ASH Annual Meeting Abstracts108(11):5341

de Lima M et al (2003) Long-term follow-up of a phase I study of high-dose decitabine, busulfan, and cyclophosphamide plus allogeneic transplantation for the treatment of patients with leukemias. Cancer 97(5):1242–1247

de Lima M et al (2007) A dose and schedule finding study of maintenance therapy with low-dose 5-azacitidine (AZA) after allogeneic hematopoietic stem cell transplantation (HSCT) for high-risk AML or MDS. ASH Annual Meeting Abstracts 110(11):3012

De Padua Silva L et al (2007) Outcome of allogeneic stem cell transplantation after hypomethylating therapy with 2-deoxy-5-azacitidine for patients with myelodysplastic syndrome. ASH Annual Meeting Abstracts 110(11):1468

Edinger M et al (2003) CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. Nat Med 9(9):1144–1150

Ferrara JL et al (2009) Graft-versus-host disease. Lancet 373(9674):1550–1561

Floess S et al (2007) Epigenetic control of the foxp3 locus in regulatory T cells. PLoS Biol 5(2):e38

Fontenot JD, Gavin MA, Rudensky AY (2003) Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 4(4):330–336

Fontenot JD et al (2005) Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity 22(3):329–341

Giralt S et al (1997) Studies of decitabine with allogeneic progenitor cell transplantation. Leukemia 11(Suppl 1):S32–S34

Jabbour E et al (2009) Low-dose azacitidine after allogeneic stem cell transplantation for acute leukemia. Cancer 115(9):1899–1905

Jacobson LO, Marks EK, Robson MJ, Gaston EO, Zirkle RE (1949) Effect of spleen protection on mortality following x-irradiation. J Lab Clin Med 34:1538–1543

Kiessling R, Klein E, Wizgell H (1975) “Natural” killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol 2(2):112–117

Kolb HJ (2008) Graft-versus-leukemia effects of transplantation and donor lymphocytes. Blood 112(12):4371–4383

Leoni F et al (2002) The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines. Proc Natl Acad Sci USA 99(5):2995–3000

Little MT, Storb R (2002) History of haematopoietic stem-cell transplantation. Nat Rev Cancer 2(3):231–238

Lorenz E et al (1951) Modification of irradiation injury in mice and guinea pigs by bone marrow injections. J Natl Cancer Inst 12(1):197–201

Lubbert M et al (2006) Non-intensive AML/MDS treatment with low-dose decitabine prior to reduced-intensity conditioning (RIC) and...
allogeneic blood stem cell transplantation of older patients. ASH Annual Meeting Abstracts 108(11):5257
McCarty J et al (2008) 5-Azacytidine prior to allogeneic transplantation effectively reduces relapse, TRM and overall mortality in high risk myelodysplasia and secondary AML [Abstract]. Bone Marrow Transplant 41(S1):S212–S213
Oliveira V et al (2008) Anti-CD4-mediated selection of Treg in vitro— in vitro suppression does not predict in vivo capacity to prevent graft rejection. Eur J Immunol 38(6):1677–1688
Pegram HJ et al (2011) Alloreactive natural killer cells in hematopoietic stem cell transplantation. Leuk Res 35(1):14–21
Pende D et al (2005) Analysis of the receptor-ligand interactions in the natural killer-mediated lysis of freshly isolated myeloid or lymphoblastic leukemias: evidence for the involvement of the Poliovirus receptor (CD155) and Nectin-2 (CD112). Blood 105(5):2066–2073
Ravandi F et al (2001) Decitabine with allogeneic peripheral blood stem cell transplantation in the therapy of leukemia relapse following a prior transplant: results of a phase I study. Bone Marrow Transplant 27(12):1221–1225
Reddy P et al (2004) Histone deacetylase inhibitor suberoylanilide hydroxamic acid reduces acute graft-versus-host disease and preserves graft-versus-leukemia effect. Proc Natl Acad Sci USA 101(11):3921–3926
Ruggeri L et al (2002) Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 295(5562):2097–2100
Sanchez-Abarca LI et al (2010) Immunomodulatory effect of 5-azacytidine (5-azaC): potential role in the transplantation setting. Blood 115(1):107–121
Tao R et al (2007) Deacetylase inhibition promotes the generation and function of regulatory T cells. Nat Med 13(11):1299–1307
Wang X et al (2010) Sequential treatment of CD34+ cells from patients with primary myelofibrosis with chromatin-modifying agents eliminate JAK2V617F-positive NOD/SCID marrow repopulating cells. Blood 116(26):5972–5982