

Epigenetic approaches in stem cell transplantation

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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

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Table 1 Procedure of allogeneic stem cell transplantation: main phases and principles

Procedure/phase	Principle
1 Conditioning regimen	
a) Cytoreductive chemotherapy	Disease control Reduction of tumour burden Induction of remission
b) Myeloablation/immunoablation	Elimination of the immune system of the recipient by (high-dose) chemotherapy or irradiation
2 Stem cell transfusion	Donation of “the new” immunosystem
3 Immunosuppression	Prevention and treatment of graft rejection and graft-versus-host disease
4 Alloreactivity as further medicinal intervention: maintenance and relapse therapy	Elimination of residual malignant cells by the graft-versus-leukaemia reaction, enhancement of the immunotherapeutic effect by donor lymphocyte infusions

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+FOXP3+ 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-1 β and IL-12 in lipopolysaccharide-stimulated human peripheral

Table 2 Epigenetic agents prior to HSCT (allogeneic haematopoietic transplantation)

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

Table 3 Epigenetic agents as relapse therapy after haematopoietic transplantation (HSCT)

Author	n	Agent	Disease	Remission	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, 5x 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)

Decitabine (Dec) and 5-azacytidine (5-Aza) in combination with HSCT or donor lymphocyte infusion (DLI)

AML acute myeloid leukaemia, MDS myelodysplasia, CML chronic myeloid leukaemia, ALL acute lymphoblastic leukaemia, CR complete remission, PR partial remission

blood mononuclear cells in vitro (Leoni et al. 2002). In bone marrow transplantation mouse model, 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).

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 á 28 days (up to 22 cycles)	3 x 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 pre-treated 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.

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