Epigenetic therapy in allogeneic hematopoietic stem cell transplantation

Qaiser Bashir1
Basem Magdy William2
Guillermo Garcia-Manero1
Marcos de Lima2

1The University of Texas MD Anderson Cancer Center, Houston, TX, USA
2University Hospitals Case Medical Center/Case Western Reserve University, Cleveland, OH, USA

Conflict-of-interest disclosure:
The authors declare no competing financial interest

Submitted: 2/14/2013
Accepted: 4/4/2013

Corresponding author:
Marcos de Lima, MD
Division of Hematology and Oncology
University Hospitals Case Medical Center
Case Western Reserve University
11100 Euclid Ave. LKS 5079
Cleveland, OH 44106. USA
Phone +1 216-983-3276
Email: marcos.delima@uhhospitals.org

DNA methylation and other epigenetic phenomena appear to be relevant in the pathogenesis of several malignant disorders. DNA methyltransferases add methyl groups to cytosine-phosphate-guanine (CpG) islands leading to gene promoter silencing. The DNA methyltransferases inhibitors azacitidine and decitabine have anti-tumor activity against a broad range of malignancies, but have been investigated mostly in myelodysplastic syndrome. In addition, these agents have immunomodulatory effects that are under investigation in the allogeneic stem cell transplantation scenario. Both drugs have been used in the perioperative period of allogeneic transplantations with varying degrees of success. It has been hypothesized that low dose azacitidine may increase the graft-versus-leukemia effect and have a role in the maintenance of remission after allogeneic transplantation for myeloid leukemias. It is also intriguing that this favorable effect might occur while mitigating graft-versus-host disease. Here we present a review of the rapidly growing field of epigenetic manipulation using hypomethylating agents in allogeneic transplantation.

Keywords: Hematopoietic stem cell transplantation; DNA methyltransferase; Leukemia, myeloid; Epigenesis, genetic; Azacitidine; Immunologic factors

Introduction

The loss of global DNA methylation was one of the earliest epigenetic abnormalities identified in cancer cells(1). Several studies have shown DNA hypomethylation as a common feature of carcinogenesis(2). On the other hand several investigators have indicated that DNA hypermethylation is a frequent phenomenon occurring across different cancer types(3). CpG island promoter methylation may affect multiple pathways involved in apoptosis, cell cycle and DNA repair, for example(6). Around 50% of tumor-suppressor genes have been reported to be silenced by aberrant DNA methylation of their promoters(6). Interestingly, DNA hypermethylation is also involved in down-regulation of tumor suppressor micro-RNAs(5,8). The mechanisms leading to aberrant DNA methylation are however not completely understood but may involve DNA instability, over expression of DNA methyltransferases(DNMTs), or environmental factors such as viral infections among others(4,7). Disruption of epigenetic mechanisms provides a target for anticancer therapies.

DNA demethylating agents not only have anti-tumor activity in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), but also appear to have immunomodulatory effects(2,9). This combination of anti-tumor activity and immune modulation along with a favorable toxicity profile makes these agents potential candidates for use in the transplantation setting.

Here we present a brief introduction to cancer epigenetics and review studies, which have evaluated the putative role of DNA demethylating agents in the allogeneic hematopoietic stem cell transplantation(allo-HSCT) setting. We do not address the potential role of histone deacetylase inhibitors in this setting, although this is another exciting avenue of investigation.

Epigenetics

Chromatin is made of basic repeating units, the nucleosomes, where packed DNA base pairs are wrapped around a histone octamer(2). Epigenetics is defined as heritable changes in gene expression caused by mechanisms other than changes in the DNA sequence, while the sum total of all epigenetic information is called the ‘epigenome’(10,11). Gene silencing through epigenetic regulation involves a coordinated interplay of a number of processes which include, DNA methylation, histone modification, and nucleosome remodeling, among others(52). For the purpose of this review, we will restrict our discussion to DNA methylation only.

DNA methylation

DNA cytosine methylation, where a methyl group is covalently bound to cytosine, occurs almost exclusively in a cytosine-phosphate-guanine (CpG) context, although non-CpG methylation has also been described, particularly in embryonic stem cells(25). Most studies have revealed that
genomic DNA methylation tends to spare CpG islands (sequences of DNA approximately 1,000 base pairs long, where the dinucleotide CpG is present at closer to its expected frequency, as opposed to other areas of vertebrate genome, where it is depleted\(^{19}\)). The majority of gene promoters are located in these islands, and DNA methylation in this location silences their activity, thus repressing gene expression\(^{11,13}\).

DNA methylation is mediated by DNMTs. This includes the maintenance of methylation by DNMT1 as well as de novo methylation during embryogenesis by DNMT3a, DNMT3b, and DNMTL\(^{14,15}\). DNMT2, on the other hand does not methylate DNA but instead is involved in methylation of aspartic acid transfer RNA\(^{16}\). In addition, recent studies have shown that DNMT3a and DNMT3b are also involved in DNA methylation maintenance\(^{17}\).

Demethylating Agents

Several therapeutic strategies have been developed to induce epigenetic changes in cancer cells. These include DNMT and histone deacetylase (HDAC) inhibitors. Although several DNMT inhibitors (DNMTis) have been studied in pre-clinical and early phase clinical trials, only two, 5-Azacitidine (Azacitidine) and 5-Aza-2’-deoxycytidine (decitabine) have been approved by the Food and Drug Administration (FDA) in the United States for the treatment of MDS\(^{2,25,26}\).

Mechanism of action of Azacitidine and Decitabine

Both azacitidine (5-Aza-CR) and decitabine (5-Aza-CdR) are prodrugs that are converted to their active triphosphate forms 5-Aza-CTP and 5-Aza-dCTP, respectively, after cellular uptake by a human concentrative nucleoside transporter 1 (hCNT1)\(^{2,25,26}\). 5-Aza-CR can be incorporated into RNA as well as DNA, whereas 5-Aza-CdR can only be incorporated into DNA\(^{25}\). The incorporation into DNA induces hypomethylation of the daughter DNA strands, while the incorporation into RNA causes ribosomal disassembly and disruption of protein translation\(^{25}\). Furthermore, it has been shown that the hypomethylating effect of decitabine is most evident at low concentrations that are effective in covalently trapping DNMT without cell-cycle arrest or cytotoxicity. At higher doses, decitabine is cytotoxic, inhibits DNA synthesis and induces cell-cycle arrest as a “classical” chemotherapy agent\(^{27}\).

Immunomodulatory effects of DNA demethylating agents

In addition to the cytotoxic effects, DNMTs appear to induce phenotypic modifications (“maturation”) of leukemic cells, including increased expression of HLA class I/II antigens and increased expression of tumor antigens. These changes, discussed below, potentially could increase susceptibility of malignant cells to immune surveillance mechanisms, such as the graft-versus-leukemia effect of allogeneic cells. In addition, DNMTi may mitigate graft-versus-host disease (GVHD) possibly by increasing the number of regulatory T cells (Tregs), or by another unknown mechanism.

Induction of terminal differentiation of leukemic blasts

Pinto et al. demonstrated the induction of morphological and functional differentiation of AML cells to mature elements following repeated exposure to decitabine\(^{28}\). Moreover, increased expression of class I human leukocyte antigens (HLAs) and HLA-DR in response to treatment with decitabine has been reported\(^{29,30}\). The increased expression of these antigens may induce a higher immunogenic potential of malignant cells thus rendering them susceptible to the graft-versus-leukemia effect (GVL) mediated by donor cells in allogeneic transplantations.

Up-regulation of major histocompatibility class 1-related chain B

Major histocompatibility (MHC) class 1-related chain A (MICA) and B (MICB) are polymorphic transmembrane glycoproteins that act as ligands for the immune complex receptor NKG2D expressed by natural killer (NK) cells, CD8 cytotoxic T-cells, and γδ-T cells. MIC is a critical component of target cell susceptibility for these cells\(^{31-33}\). Tang et al. demonstrated MICB up-regulation in cell lines following treatment with decitabine. This phenomena was accompanied by promoter DNA demethylation and DNA damage and significantly enhanced susceptibility of tumor cells to NK-cell mediated cytotoxicity\(^{34}\).

Effects on natural killer cells

Interleukin-2 (IL-2) plays an important role in the development and expansion of effector T cells and maintenance of immune tolerance\(^{34,35}\). Promotion of immune tolerance by IL-2 is mediated through the generation and maintenance of Tregs, which are generally defined by CD4+CD25+Foxp3+\(^{36-38}\). Zorn et al. demonstrated that administration of low dose recombinant IL-2 induced the expression of CD4+CD25+Foxp3+ T cells in vivo\(^{39}\). These authors further demonstrated that while low dose IL-2 therapy induced the expansion of NK cells, it did not induce Foxp3 expression in NK cells. But when NK cells were pre-treated with varying concentrations of decitabine, IL-2 induced the expression of FOXP3 suggesting that FOXP3 gene was repressed in NK cells by DNA methylation\(^{40}\). Interestingly, Chan et al. demonstrated that NK cells use DNA methylation to maintain clonally restricted expressions of highly homologous killer immunoglobulin-like receptor (KIR) genes and alleles, and that decitabine induced KIR DNA hypomethylation and heterogeneous expression of multiple KIR genes\(^{39}\).

Exploiting immunomodulatory effects of hypomethylating agents in the allogeneic transplantation setting

Possible mitigation of graft-versus-host disease by effects on regulatory T-cells

GVHD is one of the major complications of allo-HSCT and is mediated by donor T cells reacting against host antigens\(^{40}\). These T cells also play an important role in controlling a variety of critical steps after transplantation such as facilitating
engraftment of hematopoietic stem cells, immune reconstitution and elimination of residual disease, among others[41-43]. Tregs have been shown to mitigate GVHD by suppressing the early expansion of donor T cells[44].

Epigenetic modifications play a critical role in the locus coding for FOXP3, a forkhead transcription factor expressed in Tregs[37,45,46].

Sánchez-Abarca et al. demonstrated that azacitidine has profound effects on the activation and proliferation of T cells[49]. In their study, the addition of azacitidine to the cell culture significantly inhibited the activation and proliferation of T cells in a dose-dependent manner. Azacitidine-treated T cells produced significantly lower amounts of pro-inflammatory cytokines, tumor necrosis factor (TNF)-α and interferon (IFN)-γ compared with stimulated untreated T cells. Gene expression arrays revealed up-regulation of the FOXP3 and FOXO3a genes as well as genes involved in cell-cycle inhibition such as p27, p16, p53, and p73, whereas IFN and IL-10 genes were down-regulated. FOXP3 promoter methylation was decreased after prolonged drug exposure. These findings were also confirmed in vivo in a GVHD mouse model, where azacitidine treatment improved mouse survival and decreased GVHD related scores. The most effective time for the administration of the drug to prevent GVHD was in the range of 2-4 days after transplantation when the alloreactive T cell expansion is maximal. Interestingly, no significant change in FOXP3 promoter methylation pattern or azacitidine-induced increase in Tregs was seen during the first four days of culture. However, longer exposure induced significant promoter demethylation and drove T cell differentiation towards a Treg phenotype. The authors concluded that azacitidine prevents the development of GVHD by inhibiting the early expansion of T cells, while delayed and prolonged exposure minimized the risk of GVHD by Treg expansion[49].

In another study, Choi et al. demonstrated that both azacitidine and decitabine induced FOXP3 mRNA and protein expression in CD4+CD25+FOXP3+ humans (in vitro) and in mice[50]. They transplanted lethally irradiated Balb/c mice with T cell depleted bone marrow and conventional T cells along with azacitidine-treated Tregs, decitabine-treated Tregs or phosphate-buffered saline (PBS)-treated T cells. Mice receiving azacitidine or decitabine-treated Tregs became complete donor chimera, had improved survival and less clinical GVHD, compared to mice treated with PBS-treated T cells. To study the effect of the in vivo treatment of mice with demethylating agents after allo-HSCT, mice were transplanted with T cell depleted bone marrow following ablative irradiation. After recovery of the blood counts the mice were infused with MHC mismatched CD4+CD8- T cells on day +11. Mice were then treated with PBS, decitabine or azacitidine. While the mice treated with decitabine died due to excessive myelosuppression, the azacitidine-treated mice had high rates of donor engraftment and no detectable GVHD. Moreover, the authors also demonstrated maintenance of the GVL effect with azacitidine treatment. Interestingly they also indicated that decitabine treated Tregs from FOXP3 knockout mice were as suppressive as decitabine treated Tregs from FOXP3 wild-type littermate controls, suggesting that the suppressor function of decitabine or azacitidine treated Tregs is not dependent on FOXP3 expression and that expression of other candidate genes is likely modulated and/or necessary for the suppressor function of decitabine or azacitidine-treated Tregs to occur[49]. In summary, the above studies would suggest that treatment with demethylating agents may have a role in GVHD prevention[49].

Use of azacitidine to prevent and treat myeloid leukemia relapse after allogeneic hematopoietic stem cell transplantation

Demethylating agents in the treatment of acute myeloid leukemia/myelodysplastic syndrome relapse after allogeneic hematopoietic stem cell transplantation

Patients with acute leukemia or MDS that relapse after allo-HSCT have a poor prognosis. Therapeutic options for these patients are limited and the optimal management is controversial[47]. While a second allo-HSCT or donor lymphocyte infusion (DLI) offer long disease-free survival in a small subset of patients, treatment-related mortality (TRM) and relapse rates are high[48-50].

Demethylating agents have been used to treat recurrent disease after allo-HSCT, and the achievement of remission and complete donor chimerism have been reported[51,52]. Jabbour et al. treated 17 patients with low-dose azacitidine after allo-HSCT for AML/MDS as salvage (n=9) or maintenance (n=8) therapy[53]. Azacitidine was started at a median of eight months after allo-HSCT in patients with recurrent disease and at a median of two months when given as maintenance therapy. The response rate among patients with recurrent disease was 55%, but complete remission (CR) occurred mostly in indolent relapses with low bulk disease. Median CR duration in patients who received azacitidine as maintenance therapy was 17 months. The two-year event-free survival (EFS) and OS rates were 30% and 80%, respectively. Overall the drug was well tolerated and no extramedullary toxicities or increased risk of infections was noticed. In another study, Lübbert et al. treated 26 patients with AML/MDS in hematological relapse after allo-HSCT with repeated cycles of low-dose azacitidine (100 mg/day for three days) followed by DLI[54]. CR was seen in 16% of patients and another 50% had temporary disease control with stable mixed chimeras for a median duration of 72 days. Acute GVHD was seen in only three patients and the estimated two-year survival was 16%. This low survival rate was however similar to the results obtained with other interventions such as second allo-HSCT in this setting[55].

Although it is postulated that hypomethylating agents are more effective as such when given in lower doses, investigators have reported on the use of higher doses of decitabine to treat post-transplantation relapses. Ravandi et al. conducted a phase I trial of decitabine in 14 patients with advanced leukemia or transformed chronic myeloid leukemia (CML) who had failed allo-HSCT[56]. Doses were 100 mg/m2 to 150 mg/m2 given every 12 hours for five days, followed by infusion of stem cells from the original donor. The treatment was well tolerated, and disease response was seen in 57% of the patients with a median survival of 190 days. These doses were expected to be primarily cytotoxic, and it is unclear if a hypomethylating effect was present in light of the more recent knowledge discussed above.

128
Rev Bras Hematol Hemoter. 2013;35(2):126-33
Schroeder et al. showed the feasibility of prospectively administering azacitidine combined with DLI as first salvage therapy to treat relapsed AML/MDS after allo-HSCT\(^{57}\). Overall response rate was 30% and five of 30 patients achieved long-term CR. Acute and chronic GVHD were seen in 37% and 17% of the patients, respectively. Studies of demethylating agents used for the treatment of AML/MDS relapse are summarized in Table 1.

| Study                  | n  | Disease          | Agent used      | Response | PFS            | Survival          |
|------------------------|----|------------------|-----------------|----------|----------------|-------------------|
| Giralt et al.\(^{52}\) | 3  | AML/ALL          | Decitabine CR   | 100%     | -              | 1 patient alive at 160 days |
| Jabbour et al.\(^{53}\) | 17 | Relapsed disease (n = 9) | Azacitidine *RR 55% | 55% at 1 year | OS 90% at 1 year |
| Lübbert et al.\(^{54}\) | 26 | AML/CML + DLI    | Clinical benefit 66% CR 16%  | -        | OS 16% at 2 year |
| Ravanidi et al.\(^{56}\) | 14 | AML/CML + HSCT   | Decitabine RR 57% | Median PFS 60 days# | Median survival 190 days |
| Schroeder et al.\(^{57}\) | 25 | AML/MDS/CMML + DLI | Azacitidine RR 64% CR 20% | -        | Median OS 184 days |

PFS: progression-free survival; OS: overall survival; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; CR: complete remission; RR: response rate; CMML: chronic myelomonocytic leukemia; CML: chronic myeloid leukemia; HSCT: hematopoietic stem cell transplantation; MDS: myelodysplastic syndrome; DLI: donor-lymphocyte infusion

*Response rate in patients treated for relapsed disease
#For patients achieving response

### Minimal residual disease-based preemptive therapy after allogeneic hematopoietic stem cell transplantation

Detection of minimal residual disease (MRD) after allo-HSCT is associated with increased risk of hematologic relapse\(^{58-60}\). Platzbecker et al. monitored 59 patients prospectively for decreasing CD34 cell chimerism, a harbinger of impending relapse\(^{61}\). In case of decreasing chimerism, azacitidine was to be given at the standard dose of 75 mg/m\(^2\)/day subcutaneously, for seven days, for a total of four cycles every 28 days. Of the 19 patients evaluable for response one month after the 4th cycle, tenneshowed complete clearance of MRD defined as an increase of CD34\(^{+}\) donor chimerism >80%. Hematologic relapse occurred ultimately in 13 of the patients, but was delayed for approximately a median of seven months after initial decrease of CD34 donor chimerism to <80%. Grade III-IV neutropenia and thrombocytopenia were common however.

### Demethylating agents for the prevention of disease relapse after allogeneic hematopoietic stem cell transplantation

Recurrences often occur early after allo-HSCT, a period of time when bone marrow function is very susceptible to myelosuppression. If one is to propose a maintenance of remission strategy, careful determination of a “tolerable” dose is necessary since most patients may not be able to receive usually prescribed doses. In addition, lower doses may actually be associated with improved hypomethylating effects as discussed above\(^{62}\). We thus hypothesized that low-dose azacitidine would decrease relapse rates after allo-HSCT in patients with relapsed refractory AML/MDS, and treated 45 patients who had high-risk MDS/AML and were in CR at day + 30 after allo-HSCT\(^{63}\). Azacitidine was given for one to four 30-day cycles. Each cycle consisted of drug administration subcutaneously for five days, starting on the sixth week after allo-HSCT at one of five dose levels (8, 16, 24, 32, or 40 mg/m\(^2\)). DNA methylation was assessed using bisulfite pyrosequencing\(^{64}\). Azacitidine did not affect engraftment. In this dose-escalation study, the dose of 32 mg/m\(^2\) for four cycles was chosen, while thrombocytopenia prevented escalation to 40 mg/m\(^2\). At a median follow-up of 20.5 months, the one-year EFS and OS were 58% and 77%, respectively, in a cohort of mostly refractory, relapsed patients. The grade II-III and grade III acute GVHD rates were 27% and 9%, respectively. However, most acute GVHD started before starting azacitidine and patients with severe GVHD were excluded from the trial, so no firm conclusions regarding acute GVHD could be made. The probability of developing chronic GVHD was however decreased significantly with longer azacitidine treatments. Interestingly, no change in peripheral blood mononuclear cells or global DNA methylation was noticed in this study.
Goodyear et al. confirmed the tolerability of low-dose azacitidine early after allo-HSCT, and demonstrated an increase in the number of Tregs within the first three months after transplantation, as well as an induced cytotoxic CD8(+) T-cell response to several tumor antigens (such as Wilm’s tumor antigen1). Twenty-seven patients were treated with a reduced-intensity regimen and a T-cell depleted graft65.

We recently performed a matched control analysis of chronic GVHD incidence comparing patients who received low-dose azacitidine for relapse prevention, versus allogeneic HCT recipients that did not receive the drug. In this analysis, reported in abstract form only, use of azacitidine led to a significant reduction in chronic GVHD rates66.

**Use of demethylating agents prior to transplantation**

In order to assess if the use of demethylating agents may increase the toxicity of the conditioning regimen or otherwise affect transplantation outcomes, Padua Silva et al. studied 17 MDS patients that underwent allo-HSCT after prior therapy with decitabine67. Engraftment was seen in 16 patients and 100-day TRM was seen in only one patient. The authors concluded that the transplantation-related toxicity did not seem to be increased by prior use of decitabine. Others have reported similar survival after allo-HSCT in patients who did or did not receive azacitidine prior to allo-HSCT68. In addition, a trend towards decreased early relapse was seen in patients who received azacitidine prior to allo-HSCT (Table 2). The small number and the heterogeneous nature of the patients prevent a clear conclusion at this point regarding whether the use of these agents prior to allo-HSCT improves transplantation outcomes. Similarly, it is unclear if MDS patients should be transplanted at best response after azacitidine or decitabine, or after failing these drugs69.

### Table 2 - Demethylating agents used prior to allogeneic hematopoietic stem cell transplantation

| Study                        | n  | Disease                | Agent used | Response | PFS                  | OS                  |
|------------------------------|----|------------------------|------------|----------|----------------------|---------------------|
| De Padua Silva et al.67      | 17 | MDS                   | Decitabine  | CR 76%   | -                    | 64% alive at median follow-up of 12 months |
| Field et al.68               | 54 | MDS/CMML              | Azacitidine | -        | 41% at 1 year        | 47% at 1 year       |
| Lübbert et al.70             | 10 | AML/MDS/CMML          | Decitabine  | -        | 33% relapse          | 30% alive$          |
| McCarty et al.71             | 25 | MDS/AML               | Azacitidine | -        | NR at median follow-up 1 year | NR at median follow-up 1 year |
| Kim et al.72                 | 19 | MDS                   | Decitabine  | -        | NR at median follow-up 1 year | 68% at 2 year       |

PFS: progression-free survival; OS: overall survival; AML: acute myeloid leukemia; CR: complete remission; CMML: chronic myelomonocytic leukemia; MDS: myelodysplastic syndrome; NR: not reached

1 Preparative regimen; Fludarabine + Busulfan (n = 8); Fludarabine + Melphalan (n = 9)
2 Preparative regimen; Fludarabine + Busulfan (n = 54)
$ At +1, +10, +26 months after transplantation

### Table 3 - DNA demethylating agents used as part of conditioning regimen

| Study                        | n  | Disease                | Agent used + Conditioning regimen | Response | PFS                  | OS                  |
|------------------------------|----|------------------------|-----------------------------------|----------|----------------------|---------------------|
| Giralt et al.52              | 4  | CML/AMML              | Decitabine + (Bu/Cy)              | CR 50%   | -                    | 3 patients alive*   |
| De Lima et al.73             | 23 | AML/CMML/ALL/CMML     | Decitabine + Bu/Cy                | CR 91%   | Median PFS 8.9 months | Median survival 17.2 months |

PFS: progression-free survival; OS: overall survival; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; CR: complete remission; CMML: chronic myelomonocytic leukemia; CML: chronic myeloid leukemia; AMML: acute myelomonocytic leukemia; Bu: busulfan; Cy: cyclophosphamide

* At 167, 129, and 109 days post-transplantation
In the past, we investigated decitabine as part of the transplantation conditioning regimen. Decitabine was added to busulfan and cyclophosphamide, followed by HLA identical sibling donor allo-HSCT for high-risk patients with acute leukemia and chronic myelomonocytic leukemia (CMML)\(^{(73)}\). The regimen was well tolerated with a 100-day TRM of 9%. The incidence of acute and chronic GVHD was 18% and 40%, respectively. The median survival for the entire group was 17.2 months with 26% patients alive and disease free at a median of 3.3 years from transplantation. This trial, however, investigated cytotoxic doses of decitabine (Table 3) and this approach has not been pursued by other investigators.

**Future directions**

The immunomodulatory and cytotoxic effects of agents capable of epigenetic manipulation deserve further investigation in the realm of allogeneic transplantation. As an example, we and others are launching a phase I trial investigating the oral formulation of azacitidine in the prevention of relapse of AML/MDS in this setting. Other potential ideas include studies of GVHD prevention and treatment of GVHD with decitabine or azacitidine. Clinical investigators will be charged with determining efficacy and toxicities of these agents, but it is an intriguing possibility that one might be able to pharmacologically separate GVHD from GVL. This hypothesis is under active investigation in different forms and approaches by several groups around the world\(^{(19)}\).

**References**

1. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some normal cancers from their normal counterparts. Nature. 1983;301(5895):89-92.
2. Yang X, Lay F, Han H, Jones PA. Targeting DNA methylation for epigenetic therapy. Trends Pharmacol Sci. 2010;31(11):536-46.
3. Esteller M. Epigenetic gene silencing in cancer: the DNA hypermethylome. Hum Mol Genet. 2007;16 Spec No 1:R50-9.
4. Toyota M, Yamamoto E. DNA methylation changes in cancer. Prog Mol Biol Trans Sci. 2011;101:447-57.
5. Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA, et al. Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Cell. 2006;9(6):435-43.
6. Lujambio A, Ropero S, Ballestar E, Fraga MF, Setién F, et al. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Res. 2007;67(7):3492. Gitt, Anna [corrected to Git, Ana].
7. Pogribny IP, Beland FA. DNA hypomethylation in the origin and pathogenesis of human diseases. Cell Mol Life Sci. 2009;66(14):2249-61.
8. Choi J, Ritchey J, Prior JL, Holt M, Shannon WD, Deych E, et al. In vivo administration of hypomethylating agents mitigate graft-versus-host disease without sacrificing graft-versus-leukemia. Blood. 2010;116(1):129-39.
9. Sánchez-Abarca L, Gutierrez-Cosio S, Santamaría C, Caballero-Velazquez T, Blanco B, Herrero-Sánchez C, et al. Immunomodulatory effect of 5-azacytidine (5-aza-C): potential role in the transplantation setting. Blood. 2010;115(1):107-21.
10. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007;128(4):683-92.
11. Suzuki MM, Bird A. DNA methylation landscapes: provocative insights from epigenomics. Nat Rev Genet. 2008;9(6):465-76.
12. Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. Nature. 2009;462(7271):315-22. Comment in: Nature. 2009;462(7271):296-7.
13. Wang Y, Leung FC. An evaluation of new criteria for CPg islands in the human genome as gene markers. Bioinformatics. 2004;20(7):1170-7.
14. Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. Annu Rev Biochem. 2005;74:481-514.
15. Jia D, Jurkowska RZ, Zhang X, Jeltsch A, Cheng X. Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation. Nature. 2007;449(7159):248-51. Comment in: Nature. 2007;449(7159):149-9.
16. Goll MG, Kirpek A, Marmont MA, Yoder JA, Hsieh CL, Zhang X, et al. Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. Science. 2006;311(5759):395-8.
17. Jones PA, Liang G. Rethinking how DNA methylation patterns are maintained. Nat Rev Genet. 2009;10(11):805-11.
18. Lemaire M, Momparler LF, Raynal NJ, Bernstein ML, Momparler RL. Inhibition of cytidine deaminase by zebularine enhances the antineoplastic action of 5-aza-2'-deoxycytidine. Cancer Chemother Pharmacol. 2009;63(3):411-6.
19. Byun HM, Choi SH, Laird PW, Trinh B, Siddiqui MA, Marquez VE, et al. 2'-Deoxy-N4-[2-(4-nitrophenyl)ethoxycarbonyl]-5-azacytidine: a novel inhibitor of DNA methyltransferase that requires activation by human carboxylesterase 1. Cancer Lett. 2008;266(2):238-48.
20. Flotho C, Claus R, Batz C, Schneider M, Sandrock I, Ilbe S, et al. The DNA methyltransferase inhibitors azacitidine, decitabine and zebularine exert differential effects on cancer gene expression in acute myeloid leukemia cells. Leukemia. 2009;23(6):1019-28.
21. Suzuki T, Tanaka R, Hamada S, Nakagawa H, Miyata N, Design, synthesis, inhibitory activity, and binding mode study of novel DNA methyltransferase inhibitors. Bioorg Med Chem Lett. 2010;20(3):1124-7.
22. García-Manero G, Fenaux P. Hypomethylating agents and other novel strategies in myelodysplastic syndromes. J Clin Oncol. 2011;29(5):516-23.
23. Amato RJ. Inhibition of DNA methylation by antisense oligonucleotide MG98 as cancer therapy. Clin Genitourin Cancer. 2007;5(7):422-6.
24. Garzon R, Liu S, Fabbri M, Liu Z, Heaphy CE, Callegari E, et al. MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DMT3A and 3B and indirectly DMT1. Blood. 2009;113(25):6411-8. Comment in: Blood. 2009;113(25):6269-70.
25. Rius M, Stresemann C, Keller D, Brom M, Schirmer M, Keppler D, et al. Human concentative nucleoside transporter 1-mediated uptake of 5-azacytidine enhances DNA demethylation. Mol Cancer Ther. 2009;8(1):225-31.
26. Stresemann C, Lyko F. Modes of action of the DNA methyltransferase inhibitors azacitidine and decitabine. Int J Cancer. 2008;122(9):1561-7.
27. Qin T, Jelinek J, Si J, Shu J, Issa JP. Mechanisms of resistance to 5-aza-2'-deoxycytidine induces terminal differentiation of leukemic blasts from patients with acute myeloid leukemias. Blood. 1984;64(4):922-9.
28. Pinto A, Maio M, Attadia V, Faccio F, Spada OA, Di Fiore PP. 5-Aza-2'-deoxycytidine induces terminal differentiation of leukemic blasts from patients with acute myeloid leukemias. Blood. 1984;64(4):922-9.
29. Pinto A, Maio M, Attadia V, Zappacosta S, Cimino R. Modulation of HLA-DR antigens expression in human myeloid leukaemia cells. Cancer Immunol Immunother. 1980;7(4):161-6.
30. Coral S, Sigalotti L, Gasparollo A, Cattarossi I, Visintin A, Cattelan A, et al. Prolonged upregulation of the expression of HLA class I antigens and costimulatory molecules on melanoma cells treated with 5-aza-2'-deoxycytidine (5-AZA-CdR). J Immunother. 1999;22(1):16-24.
31. Tang KF, He CX, Zeng GL, Wu J, Song GB, Shi YS et al. Induction of MHC class I-related chain B (MICA) by 5-aza-2'-deoxycytidine. Biochem Biophys Res Commun. 2008;370(4):578-83.

32. Groh V, Steinle A, Bauer S, Spies T. Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. Science. 1998;279(5357):1737-40.

33. González S, Groh V, Spies T. Immunobiology of humn NKG2D and its ligands. Curr Top Microbiol Immunol. 2006;298:121-38.

34. Malek TR, Bayer AL. Tolerance, not immunity, crucially depends on IL-2. Nat Rev Immunol. 2004;4(9):665-74.

35. Nelson BH. IL-2, regulatory T cells, and tolerance. J Immunol. 2004;172(7):3983-8.

36. Zorn E, Nelson EA, Mohseni M, Porcheray F, Kim H, Litsa D et al. IL-2 regulates FOXP3 expression in human CD4+CD25+ regulatory T cells through a STAT-dependent mechanism and induces the expansion of these cells in vivo. Blood. 2006;108(5):1571-9.

37. Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity. 2005;22(3):329-41.

38. Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol. 2004;22:531-62.

39. Chan HW, Kurabo ZB, Stewart CA, Wilson MJ, Martin MP, Mace BE, et al. DNA methylation maintains allele-specific KIR gene expression in human natural killer cells. J Exp Med. 2003;197(2):245-55.

40. Kernan NA, Collins NH, Juliano L, Cartagena T, Dupont B, O'Reilly RJ. Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of graft-versus-host disease. Blood. 1986;68(3):770-3.

41. Martin PJ, Hansen JA, Buckner CD, Sanders JE, Deeg HJ, Stewart P, et al. Effects of in vitro depletion of T cells in HLA-identical allogeneic marrow grafts. Blood. 1985;66(3):646-72.

42. Curtis RE, Travis LB, Rowlings PA, Socci G, Kingma DW, Banks PM, et al. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. Blood. 1999;94(7):2208-16.

43. Ayuk F, Shimoni A, Nagler A, Schwedferger R, Kiehl M, Sayer HG, et al. Efficacy and toxicity of low-dose escalating donor lymphocyte infusion given after reduced intensity conditioning allograft for multiple myeloma. Leukemia. 2004;18(3):659-62. Comment in: Leukemia. 2004;18(9):1541-2; author reply 1542-3.

44. Edzinger M, Hoffmann P, Ermann J, Drago K, Fathman CG, Strober S, et al. CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. Nat Med. 2003;9(9):1117-8. Comment in: Nat Med. 2003;9(9):1117-8.

45. Horvath E, Nesbit ME, Kopecky KJ. Induction of donor-specific tolerance. Transplantation. 2003;76(3):268-82. Comment in: Transplantation. 2003;76(3):268-82.

46. Bousso P, Blazar BR. Immunosuppression by malignant hematopoietic cells. Blood. 2004;103(5):1635-40.

47. Anderlini P, Leibman E, Petersen FB, Buckner CD, Martin PJ, Petersen JE, et al. Azacitidine and donor lymphocyte infusions as first salvage therapy for relapsed AML after allogeneic stem cell transplantation. Blood. 2003;101(12):4671-7.

48. Radich JP, Doody JA, Kopecky KJ. Induction of donor-specific tolerance. Transplantation. 2003;76(3):268-82. Comment in: Transplantation. 2003;76(3):268-82.

49. Verdonck LF, Petersen EJ, Lokhorst HM, Nieuwenhuis HK, Dekker AW, Tilanus MG, et al. Donor leukocyte infusions for recurrent hematologic malignancies after allogeneic bone marrow transplantation: impact of infused and residual donor T cells. Bone Marrow Transplant. 1998;22(11):1057-63.

50. Collins RH Jr, Shipilberg O, Drobsky WR, Porter DL, Giralt S, Champlin R, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin Oncol. 1997;15(2):433-44. Comment in: J Clin Oncol. 1997;15(2):416-7.

51. Graef T, Kuendgen A, Fenk R, Zohren F, Haas R, Kobbe G. Successful treatment of relapsed AML after allogeneic stem cell transplantation with azacitidine. Leuk Res. 2007;31(2):257-9.

52. Giralt S, Davis M, O'Brien S, van Besien K, Champlin R, de Vos D, et al. Studies of decitabine with allogeneic progenitor cell transplantation. Leukemia. 1997;11( Suppl 1):S32-4.

53. Jabbour E, Giralt S, Kantarjian H, Garcia-Manero G, Jagasia M, Kebrabai P, et al. Low-dose azacitidine after allogeneic stem cell transplantation for acute leukemia. Cancer. 2009;115(9):1899-905.

54. Lübbert M, Häsche W, Marks R, Rüter B, Claus R, et al. Efficacy of a 3-day, low-dose treatment with 5-azacytidine followed by donor lymphocyte infusions in older patients with acute myeloid leukaemia or chronic myelomonocytic leukaemia relapsed after allografting. Bone Marrow Transplant. 2010;45(4):627-32.

55. Radich JP, Sanders JE, Buckner CD, Martin PJ, Petersen FB, Bensinger W, et al. Second allogeneic marrow transplantation for patients with recurrent leukemia after initial transplant with total-body irradiation-containing regimens. J Clin Oncol. 1993;11(2):304-13.

56. Ravandi F, Kantarjian H, Cohen A, Davis M, O'Brien S, Anderlini P, et al. 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. 2001;27(12):1221-5.

57. Schroeder T, Czibere A, Platzbecker U, Bug G, Uharek L, Tütt E, et al. Azacitidine and donor lymphocyte infusions as first salvage therapy for relapse of AML or MDS after allogeneic stem cell transplantation. Leukemia. 2013 Jan 14. [Forthcoming]

58. Mortuza FY, Papaioannou M, Moreira IM, Coyle LA, Gameiro P, Gandini D, et al. Minimal residual disease tests provide an independent predictor of clinical outcome in adult acute lymphoblastic leukemia. J Clin Oncol. 2002;20(4):1094-104.

59. Radich J, Gehly G, Lee A, Avery R, Bryant E, Edmunds S, et al. Detection of bcr-abl transcripts in Philadelphia chromosome-positive acute lymphoblastic leukemia after marrow transplantation. Blood. 1997;89(7):2602-9.

60. Radich JP, Gooley T, Bryant E, Deans C, Martin PJ, Petersen FB, et al. Second allogeneic marrow transplantation for recurrent leukemia patients “late,” 18 months or more after transplantation. Blood. 2001;98(6):1701-7.

61. Platzbecker U, Wernke M, Radic J, Kiani A, Seltman F, Rolling C, et al. Minimal residual disease (MRD) bases preemptive 5-azacytidine treatment can prevent or delay imminent relapse in patients with high risk MDS or AML after allogeneic HSCT. Results of the “RELAZA” trial. Oral presentation at: ASH Annual Meeting Abstract; 2010:116(21):679.
64. Soriano AO, Yang H, Faderl S, Estrov Z, Giles F, Ravandi F, et al. Safety and clinical activity of the combination of 5-azacytidine, valproic acid, and all-trans retinoic acid in acute myeloid leukemia and myelodysplastic syndrome. Blood. 2007;110(7):2302-8.

65. Goodyear OC, Dennis M, Jilani NY, Loke J, Siddique S, Ryan G, et al. Azacitidine augments expansion of regulatory T cells after allogeneic stem cell transplantation in patients with acute myeloid leukemia (AML). Blood. 2012;119(14):3361-9. Comment in: Blood. 2012;119(14):3199-200.

66. Lima M de, Parmar S, Chen JJ, Giralt SA, Rondon G, Popat UR, et al. Low dose azacitidin (AZA) reduces the incidence of chronic graft-versus-host disease (cGVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). Oral sessions at: ASH Annual Meeting Abstracts. 2012;120:742.

67. De Padua Silva L, de Lima M, Kantarjian H, Faderl S, Kebriaei P, Giralt S, et al. Feasibility of allo-SCT after hypomethylating therapy with decitabine for myelodysplastic syndrome. Bone Marrow Transplant. 2009;43(11):839-43.

68. Field T, Perkins J, Huang Y, Kharfan-Dabaja MA, Alsina M, Ayala E, et al. 5-Azacitidine for myelodysplasia before allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2010;45(2):255-60.

69. Jabbour E, Mathisen MS, Garcia-Manero G, Champlin R, Popat U, Khouri I, Giralt S, et al. Allogeneic hematopoietic stem cell transplantation versus hypomethylating agents in patients with myelodysplastic syndrome: A retrospective case-control study. Am J Hematol. 2013;88(3):198-200.

70. Lubbert M, Bertz H, Ruter BH, Mertelsmann RH, Finke J. Non-intensive AML/MDS treatment with low-dose decitabine prior to reduced-intensity conditioning (RIC) and allogeneic bllod stem cell transplantation of older patients. Oral session at: ASH Annual Meeting Abstract. 2006:108:5257.

71. McCarty JM, Roberts CH, Candler KS, Chung HM. Azacitidine prior to allogeneic transplantation effectively reduces relapse, TRM and overall mortality in high-risk myelodysplasia and secondary AML. Presented at: 34th Annual Meeting of the European Group for Blood and Marrow Transplantation Florence, Italy, 30 March-2 April 2008.

72. Kim DY, Lee JH, Park YH, Lee JH, Kim SD, Choi Y, et al. Feasibility of hypomethylating agents followed by allogeneic hematopoietic cell transplantation in patients with myelodysplastic syndrome. Bone Marrow Transplant. 2012;47(3):374-9.

73. de Lima M, Ravandi F, Shahjahan M, Andersson B, Couriel D, Donato M, et al. 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. 2003;97(5):1242-7.

74. Reddy P, de Lima M, Koreth J. Emerging therapies in hematopoietic stem cell transplantation. Biology of blood and marrow transplantation. Biol Blood Marrow Transplant. 2012;18(1 Suppl):S125-31.