Genomic Loss of Mismatched Human Leukocyte Antigen and Leukemia Immune Escape From Haploidentical Graft-Versus-Leukemia

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Recent developments in cell processing and immunosuppressive strategies has allowed the safe infusion of high numbers of donor T cells in the context of clinical haploidentical hematopoietic stem cell transplantation (HSCT). Haploidentical T cells display an intrinsic ability to recognize and eliminate residual patient leukemic cells, largely due to alloreactivity against the patient-specific human leukocyte antigen (HLA) molecules encoded on the mismatched haplotype. However, recent evidence has shown that leukemia, like many other tumors displaying pronounced genomic instability, is frequently able to evade this potent graft-versus-leukemia effect by undergoing de novo genomic mutations, which result in the permanent loss of only those HLA molecules targeted by haploidentical donor T-cell alloreactivity. This review summarizes the recent clinical and experimental evidence regarding this phenomenon, and its therapeutic and clinical consequences.

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Ever since the landmark demonstration of the clinical feasibility of haploidentical hematopoietic stem cell transplantation (HSCT), it has been clear that stringent control of donor T-cell alloreactivity is a key issue determining clinical outcome in this particular context of transplantation. Although extensive depletion of donor T cells from the graft could efficiently prevent the risk of uncontrollable graft-versus-host disease (GvHD), concerns were focused on how transplanted patients could control opportunistic infections and residual malignant cells.

A breakthrough finding was the demonstration, in the context of T-cell–depleted haploidentical HSCT, of the beneficial anti-leukemic role of natural killer (NK) cells that, in the absence of competing T cells, rapidly reconstitute a repertoire shaped on the donor’s genetic background. In approximately 50% of cases, the human leukocyte antigen (HLA) types and killer cell immunoglobulin-like receptor (KIR) assets of donor-recipient pairs configure what has been defined as “predicted NK alloreactivity.”2-4 This condition, when present, has been associated with a drastic reduction in the incidence of disease relapse in patients with acute leukemia transplanted in disease remission.2,3,5 Still, besides the unmet issue of immune protection against opportunistic infections, patients either lacking a suitable NK alloreactive donor or with active disease at time of transplantation experienced an unacceptable rate of disease relapses and suffered a dismal overall outcome after T-cell–depleted HSCT.6

Thus, over recent years, many groups have focused their attention on the development of novel strategies to allow the infusion of donor T cells in the haploidentical context. Such strategies include ex vivo cellular and genetic manipulation of T cells,7-9 as well as the in vivo use of immunosuppressive drugs,10 as reviewed in the present issue of Seminars in Oncology. Many of these approaches show promising results in terms of disease control, suggesting an active role of donor T cells in the elimination of residual leukemic cells, and prompting investigations on the biological bases of this effect.

T-CELL ALLORECOGNITION OF MISMATCHED HLA MOLECULES IN HSCT

The ability of the immune system to discriminate between self and non-self is inherent to its protective function against immunogenic pathogens, which are presented as antigenic peptides to T cells educated in
the thymus to be restricted for self-HLA molecules.\textsuperscript{11} Intriguingly, a large number of self HLA-restricted T cells (up to 10%) also have the ability to recognize non-self HLA molecules when encountered in the context of pregnancy or allogeneic tissue grafting.\textsuperscript{12} This can occur both by direct recognition of the alloantigen in complex with largely undefined allopeptides, or by indirect recognition of peptides processed from polymorphic regions of the allogeneic HLA molecules presented by self-HLA restriction elements.\textsuperscript{13} The latter mechanism is also at the basis of T-cell alloreactivity towards minor histocompatibility antigens (mHags), polymorphic peptides derived from antigens encoded anywhere in the human genome and presented by self-HLA restriction elements.\textsuperscript{14} In contrast to the limited number, a few dozen, of mHags identified to date at the molecular level, several thousands of different HLA class I and class II variants have been described (http://www.ebi.ac.uk/imgt/hla), reflecting the fact that the major histocompatibility complex (MHC) is the most polymorphic gene complex known in eukaryotes.

Since the beginnings of allogeneic HSCT in the 1960s, it has become increasingly clear that T-cell alloreactivity towards mismatched HLA and mHags represents an important barrier to successful transplantation, because it is at the basis for two major clinical complications associated with the procedure, graft rejection and GvHD.\textsuperscript{15} On the other hand, a certain degree of “mild” alloreactivity is needed to allow for immune control of residual leukemia by donor T cells, as evidenced by significantly increased relapse rates in transplants from monozygotic twins lacking any mHag mismatches, compared to HLA-identical siblings, as well as in transplants performed in the absence or in the presence of T-cell depletion.\textsuperscript{16,17} T-cell alloreactivity mediating the beneficial graft-versus-leukemia (GvL) effect is indeed directed against mHags and, in the context of partially HLA-mismatched HSCT, against one or several HLA antigens from different loci. Given the considerably higher frequency of alloreactive T-cell precursors against HLA alloantigens compared to mHags, it is implicit that the chances of GvL, but also of its downside GvHD, will be greater in partially HLA-mismatched compared to perfectly HLA-identical HSCT. In line with this notion, it has been shown that the risk of leukemia relapse after matched unrelated donor (MUD) HSCT is significantly reduced by the presence of HLA-DPB1 mismatches between patient and donor,\textsuperscript{18} a condition occurring in more than 80% of unrelated transplants. Interestingly, it also was shown that this protective effect can be dissected at least partly from severe acute GvHD by applying an innovative HLA-DPB1 T-cell epitope matching algorithm, which allowed us to determine permissive HLA-DPB1 mismatches associated with significantly reduced relapse rates but similar rates of severe acute GvHD compared to HLA-DPB1 allele matches after MUD HSCT.\textsuperscript{19} Additional studies are warranted to identify similar strategies for other HLA loci. In particular, in the context of haploidentical HSCT, no indication is available to date for particularly favorable or unfavorable mismatch combinations. However, it can be assumed that the driving force of T-cell alloreactivity against mismatched HLA alleles from an entire haplotype should be a potent weapon against relapsing leukemia, a notion that is supported by recent findings from our group and from others concerning HLA loss immune escape mechanisms in this context, as outlined below.

**ALTERATIONS IN THE ANTIGEN PROCESSING AND PRESENTATION MACHINERY IN CANCER**

The down-modulation of HLA class I molecules on the cell surface is the most documented mechanism by which tumor cells avoid recognition by the immune system. Alterations in HLA class I expression are a widespread finding in most tumors analyzed to date. The rates of HLA class I loss in some tumors are near 100%; for example, the rate is 96% in cervix carcinomas, 96% in breast carcinomas, 87% in colorectal carcinomas, and 70% in laryngeal carcinomas.\textsuperscript{20} These data have considerable intuitive appeal, suggesting that down-regulation or loss of HLA class I molecules can confer a selective advantage to tumor cells by enabling them to avoid recognition by CD8\(^+\) T lymphocytes, a concept supported also by evidence from different animal models of solid tumors.\textsuperscript{21}

Several different mechanisms underlying down-regulation or loss of HLA class I by human tumors have been described, resulting in a number of different phenotypes.\textsuperscript{22} Total loss of HLA class I expression may be due to mutations in the \(\beta_2\)-microglobulin (\(\beta_2\)m) gene, which encodes a protein necessary for the stable expression of HLA class I molecules on the cell surface, or to mutations in the antigen-presenting machinery, such as the down-regulation of the proteosome multicatalytic complex subunits LMP-2 and LMP-7 and of peptide transporters TAP-1 and TAP-2.\textsuperscript{23} Loss of an entire HLA haplotype has been described in different tumors and tumor cell lines with variable frequency,\textsuperscript{24} and may occur through deletions or acquired uniparental disomy (aUPD) of chromosome 6p21, a genomic alteration that will be further described in the next section of this review. Locus-specific down-regulation is often transcriptional, caused by oncogenes with transcription factor activity such as Ras, c-myc, or HER-2/neu, or by viral products such as human papillomavirus (HPV) E6, and the levels of HLA mRNA and cell surface molecules can frequently be recovered by culturing the tumor cells in the presence of interferons.\textsuperscript{25} Finally, allele-specific loss of HLA class I antigens in tumors can be mediated by structural alterations in the class I heavy chain, and has been described in particular in colon carcinoma, melanoma, and cervical cancer.\textsuperscript{25}
All the mechanisms detailed above point to the fact that tumors may adopt strategies to evade surveillance from the immune system. However, the most stunning evidence that tumors may actively use these mechanisms to avoid being targeted by a specific immune response comes from clinical studies of adoptive immunotherapy for melanoma, with the description of relapsing melanoma lesions harboring de novo genomic loss of HLA due to down-regulation of β2m and of a stringent correlation between responsiveness to T-cell immunotherapy and HLA class I expression levels in individual lesions.26,27

Interestingly, several reports were concordant in demonstrating that genomic and phenotypic alterations in HLA are a rare finding in leukemia,28,29 hinting towards a relevant difference from solid tumors and possibly explaining the marked sensitivity of hematologic malignancies to immune manipulations. Among others, of particular interest is a report by Masuda and collaborators,29 showing that loss or down-regulation of HLA expression at initial diagnosis is rare (3/39; 7.7%) but may become more frequent in leukemia relapsing after chemotherapy, since it was documented in two of five cases analyzed (40.0%).

ACQUIRED UNIPARENTAL DISOMY AND LOSS OF MISMATCHED HLA IN LEUKEMIA AFTER HAPLOIDENTICAL TRANSPLANTATION

One of the mainstays in the treatment of acute leukemia is the accurate monitoring of minimal residual disease (MRD) molecular markers over time, which is necessary to tailor therapeutic intervention. After myeloablative allogeneic HSCT for high-risk leukemia, hematopoietic chimerism (HC) is considered a valid surrogate marker for MRD, standardized and available for virtually every patient.30

Still, the most commonly used techniques for HC monitoring in clinical practice, based on polymerase chain reaction (PCR) detection of specific DNA repeat polymorphisms (VNTR [variable number tandem repeat] and STR [short tandem repeat] analyses), display a limited sensitivity of 1%-5%, granting little improvement compared to morphologic bone marrow examination. Since by definition in haploidentical HSCT patient and donor present one or more HLA mismatches, in our center we validated low-resolution genomic HLA typing of bone marrow aspirate samples as an alternative technique for HC assessment. This method displayed an improved sensitivity and specificity as compared to STR techniques,31 prompting us to implement both assays in our practice, performed in parallel to grant maximal sensitivity of relapse detection.

However, an unexpected observation came to our attention, with several cases of false negative results for the genomic HLA HC assay in overt hematologic relapse, prompting further investigation. Thus, we purified the leukemic blasts harvested at relapse in these patients and performed genomic HLA typing also on the sorted subpopulation, demonstrating the selective genomic loss of the mismatched, patient-specific HLA haplotype in the leukemic blasts32 (Figure 1).

By taking advantage of whole-genome single-nucleotide polymorphism (SNP) arrays, we could demonstrate that the genetic mechanism at the basis of these de novo mutant variants was represented by loss of heterozygosity (LOH) in an extensive region of chromosome 6p, encompassing the whole MHC. LOH in this region was present in the absence of SNP copy number variations (CNVs), formally demonstrating that genomic loss of the mismatched HLA haplotype was due to de novo aUPD. The molecular events at the basis of this type of genomic abnormality are uncertain, but it has been postulated that aUPD may derive either from mitotic homologous recombination events, or from an attempt to correct for the unbalanced loss of chromosomal material by using the remaining alleles as a template. aUPD cannot be detected by standard cytogenetics, and for this reason has only recently been characterized as one of the possible molecular mechanisms underlying LOH. Nonetheless, numerous reports have already demonstrated a very high frequency of aUPD in many tumors, including hematologic malignancies.35 Its occurrence can promote tumor cell survival or progression by various mechanisms, including an increase in the allelic burden of different oncogenes, the loss of the second allele of a tumor suppressor gene (Knudson’s “second hit”), or, in our specific case, the genomic loss of an immunodominant target of T cells.

The latter phenomenon, which was documented in 5 of 17 relapses after haploidentical HSCT in our series, was soon confirmed in an independent report by Vilalobos et al, describing two cases of HLA loss by chromosome 6p aUPD amongst three pediatric patients with acute myeloid leukemia (AML) who relapsed after haploidentical transplantation.34

Importantly, both reports concomitantly demonstrated ex vivo that loss of the mismatched HLA haplotype completely abolished the recognition and elimination of AML blasts by donor T cells, either freshly harvested or circulating in the patient after transplantation.32,34 Thus, chromosome 6p aUPD configures an extremely effective mechanism of immune escape from the antileukemic immune response after transplantation, allowing the outgrowth of the leukemic clone harboring this mutation. This observation has relevant clinical consequences: not only does it demonstrate that the donor-derived T cells circulating in the patient at the time of relapse become inefficient bystanders, but also that any attempt to induce remission by infusion of T lymphocytes freshly harvested from the same donor, the common clinical practice of post-transplantation donor lymphocyte infusions (DLIs),35 is expected to be futile against the leukemic
Figure 1. Loss of mismatched HLA in leukemic cells after haploidentical HSCT. (A) Biological basis and consequences of genomic loss of the patient-specific HLA haplotype after transplantation. Leukemic cells, heterozygous at diagnosis (on the left), are subject to an intense immunologic stress after transplantation, mostly mediated by donor T cells targeting the mismatched HLA haplotype (in red). This pressure, combined with the genomic instability intrinsic to tumor cells, leads to the generation and selection of mutant variants that lack the patient-specific HLA haplotype (on the right) and therefore are no longer recognized by donor T lymphocytes. (B) Schematic representation of low-resolution HLA typing by forward PCR sequence-specific oligonucleotide probing (SSOP) of DNA from a donor and a haploidentical patient’s leukemia at diagnosis or at HLA loss relapse after transplantation. The lines on each strip represent signals of probes specifically hybridizing to the HLA alleles amplified from the DNA under analysis. Signals for HLA alleles from the shared haplotypes are shown in black, signals for HLA alleles from the patient- and the donor-specific haplotype are shown in red and blue, respectively. Note the absence of patient-specific signals in the purified leukemic blasts at relapse, demonstrating genomic loss of the corresponding allele. (C) SNP profile of chromosome 6 of purified AML blasts harvested at diagnosis (upper dot plots) and at relapse after haploidentical HSCT (lower dot plots) (adapted from Toffalori et al46). Upper plots show the B allele frequency, which indicates the zygosity of each SNP: a physiological situation that comprises equal representation of AA, AB, and BB genotypes, respectively, displaying B allele frequencies of 0, 0.5, and 1, as observed in blasts at diagnosis. Lower plots show the LogR ratio, which is a measure of SNP CNV, with a normal value defined as 0, as at diagnosis. Note in AML at relapse the large region of the short arm of chromosome 6 without heterozygous SNPs (absence of dots with B allele frequency 0.5) but with conserved CNV, demonstrating de novo aUPD encompassing the MHC (in yellow).
cells, and potentially harmful to the patient due to the conserved risk of inducing GvHD). These considerations prompt the investigation of alternative strategies to treat these peculiar variants of leukemia relapse, which will be the topic of the final section of this review.

An even more clinically relevant question is whether it is possible to predict, and prevent, the occurrence of HLA loss relapses in transplanted patients. Directly related to this issue is the question of when and how the mutant leukemic clone arose. In other terms, were the leukemic cells harboring genomic HLA loss already present at the time of diagnosis as a minor subclone, and then expanded upon immune pressure, or did the mutation occur de novo after transplantation, prompted for instance by DNA damage during patient conditioning and/or by the inflammatory cytokine milieu? LOH encompassing the MHC has been reported to occur occasionally (possibly in up to 3% of cases) in leukemia at diagnosis, leading to HLA mistypings of blood samples containing a high amount of leukemic blasts.\(^{36,37}\) From a biological standpoint this observation suggests that, at least theoretically, some of the leukemia with chromosome 6 aUPD observed after haploidentical HSCT could have arisen from a clone already present at diagnosis. However, several lines of evidence suggest that most, if not all, HLA loss relapses may arise from de novo mutations occurring after transplantation. First, since chromosome 6 aUPD at diagnosis is expected to affect either of the two haplotypes with similar frequency, we would have to envisage a very high frequency of this alteration at diagnosis to explain its presence in 40% of relapses after haploidentical HSCT, all targeted to the haplotype mismatched between donor and recipient. Conversely, SNP array analysis of large series of patients has shown it to be a rare event in AML at diagnosis\(^{38,39}\) or at relapse in patients treated solely with chemotherapy,\(^{40}\) at least 10 times less frequent than in patients relapsing after haploidentical HSCT. Second, HLA loss relapses arising from pre-existing mutant clones would be expected to overcome immune control and outgrow more quickly as compared to their classical counterparts. Conversely, in our experience HLA loss relapses tend to occur at later time-points as compared to other relapses, suggesting the existence of a considerable phase of equilibrium between the transplanted immune system and the residual leukemic cells, an equilibrium that is eventually broken by the de novo arousal of the mutant leukemic progeny, which subsequently is able to rapidly outgrow.

Although this evidence seems to suggest that most of HLA losses at relapse are de novo events, those rare cases of chromosome 6p aUPD that occur at diagnosis also have relevant clinical implications: beside the aforementioned risk of mistyping, the possible occurrence of these leukemic variants should be considered when partially HLA mismatched HSCT is the selected therapeutic option, to preferentially choose donors that are expected to be alloreactive against the HLA alleles retained by the mutant leukemia.

**GENOMIC HLA LOSS BY ACQUIRED UPD IN OTHER TRANSPLANTATION SETTINGS AND PATHOLOGICAL CONDITIONS**

As outlined above, HLA molecules mismatched between patient and HSCT donor represent potent targets of T-cell alloreactivity. Immunologic pressure is therefore expected to be highest in the context of haploidentical HSCT, in which an entire haplotype can act as target for donor T cells, granting a substantial selective advantage to leukemia cells that have selectively lost the relevant haplotype. Over recent years, HLA mismatches increasingly also have been allowed for transplantation from volunteer MUDs, for which donor-recipient compatibility for 8/8 of the HLA-A, B, C, DRB1 alleles is considered to be the gold standard. In contrast, mismatching for so-called low-expression alleles including HLA-DRB3/4/5, DQ and DP was shown not to significantly impact the final outcome of transplantation and is therefore omitted for MUD selection. Moreover, for the 50% of patients who do not find an 8/8 matched MUD in acceptable time, additional mismatches at one or more of the four classical loci are also accepted.\(^{41}\) Finally, an alternative to haploidentical HSCT for patients unable to find an acceptable MUD is transplantation from umbilical cord blood donors (UCB), in which the matching requirements are far less stringent.\(^{42}\)

Does HLA loss occur also in these contexts, and with what incidence? This question is not of trivial relevance, in particular if we consider that to date haploidentical HSCT is mostly performed in few specialized centers, whereas MUD HSCT is common practice worldwide, with an approximately 10 times higher number of MUD compared to haploidentical transplants performed every year both in Europe\(^{43}\) and in the United States (www.marrow.org).

In the context of a systematic comparison between the whole genome SNP profile of 21 AML samples at diagnosis with their counterparts at relapse after MUD-HSCT, Waterhouse et al described two cases of de novo chromosome 6p aUPD.\(^{44}\) These data demonstrate that this phenomenon can be of relevance also in the MUD setting, and suggest an incidence of approximately 10% of the total relapses after MUD HSCT, about three times less than after haploidentical transplantation. In line with these observations, we recently reported on a case of HLA loss following two subsequent transplants from unrelated donors presenting only with isolated mismatches at HLA-C and HLA-DPB1, followed by serial DLIs.\(^{45}\) Our findings show how even in the context of a
limited number of HLA mismatches between the donor and the patient, continuous immune pressure can elicit the outgrowth of HLA loss immune escape variants. Larger studies are warranted to better define the incidence of this phenomenon in the MUD setting. Still, we would already call for caution in the use of DLIs to treat relapse after MUD HSCT, and suggest a careful assessment of the genomic HLA typing of the relapsing leukemia before initiating potentially ineffectual lymphocyte infusions.

Of note, a recent report from Stolzel and colleagues described a case of de novo chromosome 6p aUPD in an extramedullary myeloid sarcoma occurring after HLA-identical sibling HSCT. In this context, the loss of a HLA haplotype may confer a putative immune advantage to leukemic cells by abrogating the appropriate restriction elements for tumor antigens and minor histocompatibility antigens. Conversely, the possible association between genomic loss of HLA and the gain of tropism for an immune-privileged extramedullary site, another frequent mechanism to avoid antitumor immunity, is trickier to speculate upon. Are the two events biologically connected? Which one occurred first? Which was the most relevant in determining relapse? Again, larger studies characterizing in detail the features of leukemia relapses after allogeneic transplantation are warranted to provide clues to these issues.

And what about other diseases? To our knowledge, no report to date has described immune escape by HLA loss in lymphoid malignancies, and so far we did not come across this phenomenon in patients presenting with relapse of acute lymphoblastic leukemia (ALL) after partially HLA-mismatched HSCT at our center. A single case of de novo chromosome 6p aUPD in ALL has been reported by Isoda et al, who showed that this condition allowed an exceptional event of maternofetal transmission of ALL during pregnancy. This suggests that, in this context, loss of the mismatched HLA molecules conferred to the ALL blasts the ability to evade protective anti-host immunity in the child.

Finally, de novo chromosome 6p aUPD has been suggested to play a role in a completely different disease context, as reported in a recent study coordinated by Katagiri and coworkers on behalf of the Japan Marrow Donor Program. These investigators evidenced that chromosome 6p aUPD is the most common genomic alteration in the hematopoietic cells circulating in patients affected by acquired aplastic anemia, a non-malignant disease in which autoimmunity targets marrow progenitors. They showed that a number of specific HLA alleles are preferred targets of autoimmunity, prompting the generation of “escape variants” of hematopoietic progenitors presenting with selective loss of the relevant alleles.

**FUTURE PERSPECTIVES: EARLY DIAGNOSIS AND TARGETED THERAPY OF HLA LOSS RELAPSES**

The best therapeutic approach for leukemia relapses after allogeneic HSCT is a strongly debated issue, with none of the available options providing satisfactory results. Still, the most widely adopted approach, at least in the context of HLA-matched HSCT, is based on the infusion of donor lymphocytes to boost the donor anti-leukemic immunity with fresh effector T cells. While this approach has shown a clear benefit in the context of chronic myeloid leukemia, its efficacy in curing overt relapse of a rapidly growing disease such as acute leukemia, is much less documented. Thus, consensus is growing about the importance of timely intervention, by “pre-emptive” treatment of impending leukemia recurrence, guided by molecular markers of MRD.

How can this trend be conciliated with the evidence of frequent relapses that, by losing the mismatched HLA, become insensitive to donor T cells? The answer must be sought in how, and when, we can ascertain the diagnosis of genomic HLA loss.

The currently available methods to demonstrate genomic loss of HLA in relapsing leukemia are based on molecular HLA typing techniques, which are able to detect host-derived subpopulations with a sensitivity of 1% at best. Thus, even in cases with clear evidence of disease relapse, detected by molecular or flow cytometry techniques, which have a higher sensitivity, a negative result from HLA typing of the whole bone marrow specimen is not reliable if the pathological subset is small. A possible solution is resorting to magnetic or flow cytometry–based purification of the leukemic blasts for subsequent HLA typing. Although effective, and often necessary to confirm the suspect of HLA loss, also this approach can be performed only at overt hematologic relapse and is cumbersome to implement in routine clinical practice, requiring the collection of large quantities of viable bone marrow material at each post-transplantation follow-up.

Thus, the development of new molecular assays able to sensitively assess the presence of leukemic cells and their HLA genomic assets in a single test would represent a considerable improvement, allowing the timely distinction between “classical” relapses, which would possibly be promptly treated with DLIs, and HLA loss relapses.

What should be the treatment of the cases with documented HLA loss? Based on the net result of the genomic alteration, two possible alternative immunotherapeutic strategies can be envisaged (Figure 2). The first is a second transplantation, from an “alternative” haploidentical stem cell donor, selected for being mismatched against the HLA haplotype retained by leukemic blasts after the UPD event. Theoretically, this
would grant the advantage of a 50% incompatibility between the donor and patient healthy tissues, and at the same time full incompatibility between the donor and leukemia. Moreover, given that chromosome 6p aUPD results in the genomic loss of one HLA haplotype, the mutant leukemic cells would be genetically unable to undergo a further UPD event to escape the alloreactive immune response from the second donor’s T cells. Although this treatment approach is promising, as demonstrated by the evidence of patients alive and in remission at long-term follow-up after a second HSCT at our center, its main limit resides in the treatment toxicity it implies, especially in patients that need a debulking chemotherapy before transplantation. Again, the prospect of an early molecular diagnosis of HLA loss relapse would possibly allow us to enroll patients to a second HSCT sooner, sparing them salvage chemotherapy and possibly limiting transplant-related mortality.

A second possible approach that could be envisaged is based on the use of donor NK cells: leukemic cells that undergo genomic loss of one HLA haplotype in several cases also lose the ligands for donor inhibitory KIRs, becoming in principle susceptible to NK cell alloreactivity. This event is not sufficient to prevent clinical disease relapse in vivo, possibly due to the rapid growth rate of the relapsing leukemia and to the tolerizing milieu established in transplanted patients. Still, the possibility of infusing high numbers of purified donor NK cells in case of HLA loss relapse is worth investigating. Clinical experience with NK cell infusions in transplanted patients is limited, but preliminary data showed feasibility and low risk of inducing GvHD. Further trials are needed to establish the doses, in vivo or ex vivo stimulation protocols, and efficacy of this approach.

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