haematologica
Journal of the Ferrata Storti Foundation

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by Michael Uhlin, Linnea Pettersson and Emelie Foord

Received: October 13, 2026.
Accepted: November 18, 2026.

Citation: Michael Uhlin, Linnea Pettersson and Emelie Foord. Now you see it, now you don't: bringing human leukocyte antigen-loss relapse into clinical practice.
Haematologica. 2026 Nov 26. doi: 10.3324/haematol.2026.302086 [Epub ahead of print]

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Now you see it, now you don’t: bringing human leukocyte antigen-loss relapse into clinical practice

Michael Uhlin[1]*, Linnea Pettersson[1], Emelie Foord[1]
1) Karolinska Institutet, Department of Medicine, Stockholm, Sweden

*Corresponding author. Email: michael.uhlin@ki.se

Author contributions: MU conceived the editorial. LP and EF contributed to the review and finalization of the editorial. All authors approved the final version of the manuscript.

Disclosures: MU, LP and EF are employees of Devyser AB.

Allogeneic hematopoietic cell transplantation (allo-HCT) represents one of the most successful forms of cancer immunotherapy. Its curative potential relies substantially on the graft-versus-leukemia effect, in which donor-derived immune cells recognize and eliminate remaining malignant cells. However, the same immune pressure that controls disease can also shape its evolution. Relapse after allo-HCT should therefore not be regarded simply as regrowth of residual malignant cells, but also as a process of immune selection in which surviving clones may be selected for, or acquire, mechanisms that allow them to escape donor immunity.

A particularly striking example is genomic loss of mismatched human leukocyte antigen (HLA). In 2009, Vago and colleagues demonstrated that leukemic cells at relapse after haploidentical transplantation could acquire copy-neutral loss of heterozygosity of chromosome 6p, resulting in loss of the mismatched HLA haplotype without loss of overall HLA class I expression. The consequence was profound: donor T cells recognized and killed the original leukemic cells but failed to recognize the HLA-loss variants [1].

Subsequent studies established that HLA-loss is a recurrent and clinically relevant mechanism of immune escape following allo-HCT. Crucitti *et al*. identified HLA-loss-related relapse in 23 out of 69 relapses after mismatched related donor transplantation, corresponding to approximately one third of relapses in this setting. HLA-loss was particularly associated with myeloid malignancies and tended to occur later than conventional relapse [2]. Moreover, Toffalori and colleagues demonstrated that the same mechanism could occur after mismatched unrelated donor transplantation, broadening its relevance beyond the original haploidentical setting [3]. In a recent multicenter study spanning 533 relapses of hematological malignancies, Fleischhauer *et al.* identified a varying incidence of HLA loss across donor settings, being identified in 28.7% of relapses after haploidentical

transplantation and 7.2% after unrelated-donor transplantation [4]. Together, these findings establish HLA loss as a clinically relevant mechanism of relapse across different donor settings.

The wider concept of post-transplant immune escape has since become increasingly evident. Christopher *et al.* showed that acute myeloid leukemia (AML)-associated relapse after allo-HCT was not characterized by recurrent acquisition of new immune-related driver mutations. Instead, malignant cells frequently displayed altered expression of immune-response pathways, most notably downregulation of MHC class II molecules. In 17 out of 34 evaluated patients, MHC class II expression was reduced at relapse, and leukemic cells with this phenotype showed a markedly diminished capacity to activate allogeneic CD4+ T cells [5]. These observations support the concept of immunoediting, whereby transplantation creates a selective environment in which malignant populations capable of evading immune recognition are preferentially retained and may continue to expand [6].

In this issue of *Haematologica*, Kimura and colleagues extend the clinical spectrum of HLA-loss-related relapse by describing the mechanism in a case of adult T-cell leukemia/lymphoma (ATL) after haploidentical allo-HCT [7]. Importantly, they also demonstrate a feasible approach to its detection. Using the characteristic ATL phenotype to identify malignant cells, the authors showed selective loss of the recipient-specific mismatched HLA allele by allele-specific flow cytometry, while expression of an HLA allele encoded on the shared haplotype was preserved. Targeted sequencing of sorted ATL cells subsequently confirmed loss of heterozygosity involving the mismatched HLA haplotype.

This observation is important beyond the described individual case. HLA-loss-related relapse has predominantly been discussed in AML and other myeloid malignancies. The present report suggests that it should instead be viewed as a consequence of HLA-directed immune pressure that may occur across hematological malignancies when the necessary selective conditions are present.

Growing recognition of the diagnostic relevance of HLA-loss has been accompanied by expanding capabilities for its detection. Current molecular methods can identify HLA-loss with increasing sensitivity. Targeted PCR-based approaches have demonstrated that HLA-loss-related relapse can be detected rapidly and reliably [8]. More recently, next-generation sequencing (NGS)-based approaches using markers located across the HLA region have enabled detection without requiring prior knowledge of a specific HLA mismatch and can be combined with conventional chimerism analysis, making this a valuable tool for relapse monitoring [9].

However, the approach used by Kimura *et al.,* using phenotype-directed flow cytometry has an important advantage in this situation; HLA expression can be examined directly within the malignant population. This approach may therefore provide a rapid first-line strategy when an informative allele-specific antibody and a clearly identifiable tumor phenotype are available. Molecular methods remain

important for confirmation and for cases in which suitable antibodies are lacking. Rather than competing technologies, flow cytometry and molecular assays may therefore represent complementary approaches to the same clinical question, evaluating HLA-loss at the phenotypic and genomic level.

The clinical impact of identifying HLA-loss is significant. Donor lymphocyte infusion (DLI) aims to reinforce donor-derived alloreactivity against recurrent malignant cells. However, once the relevant mismatched HLA molecules have been lost, lymphocytes from the original donor are biologically unlikely to restore an effective graft-versus-leukemia response, while the risk of graft-versus-host disease remains [4]. In the current study, identification of HLA-loss directly contributed to withholding a planned DLI [7], demonstrating the clinically utility of HLA-loss testing. Knowledge of HLA-loss status may similarly influence and inform suitable donor selection for a second transplantation.

Despite these potential clinical implications, a substantial gap remains between the knowledge on HLA-loss biology and clinical implementation. In a recent international survey of 62 transplant physicians from 11 countries, HLA-loss testing was performed before DLI in only 17.7% of cases and before second transplantation in only 21%. Limited assay availability, cost and turnaround time were major barriers [10]. This is striking considering that the mechanism has been recognized for more than 15 years and that increasingly accessible diagnostic approaches are now available, facilitating its translation into clinical practice.

The report by Kimura *et al.* is therefore timely. It broadens the disease spectrum associated with HLA-loss-related relapse and illustrates how established laboratory technologies can be adapted to recognize immune escape rapidly. The next challenge is no longer simply to prove that HLA-loss exists, but to make testing sufficiently accessible and become an integrated part of routine relapse monitoring after HLA-mismatched allo-HCT.

Ultimately, identifying that a patient has relapsed is only the beginning. The increasingly important question is how the malignant cells were able to escape - because the answer may guide what treatment still has a chance to work.

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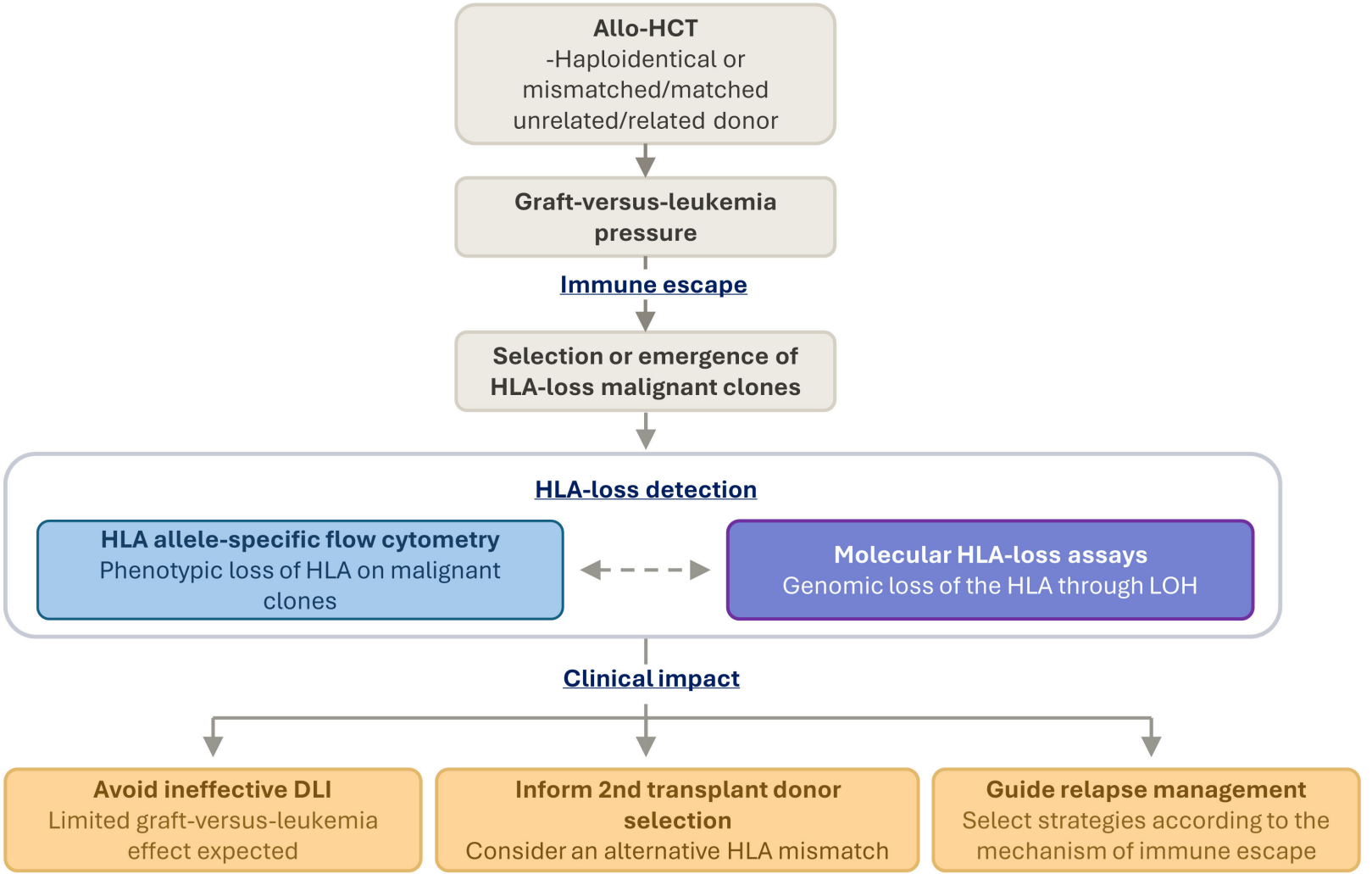
Figure 1. Detection of HLA-loss-associated relapse after allo-HCT. HLA-loss can be detected phenotypically by allele-specific flow cytometry within the malignant population, and/or genomically by molecular assays targeting the HLA region using for example NGS. The two are complementary rather than competing providing information at phenotypic and genomic level respectively and can also be performed sequentially. Confirmed HLA-loss argues against DLI from the original donor and informs donor selection and relapse management.


Allo-HCT
-Haploidentical or mismatched/matched unrelated/related donor
Graft-versus-leukemia pressure
Immune escape
Selection or emergence of HLA-loss malignant clones
HLA-loss detection
HLA allele-specific flow cytometry
Phenotypic loss of HLA on malignant clones
Molecular HLA-loss assays
Genomic loss of the HLA through LOH
Clinical impact
Avoid ineffective DLI
Limited graft-versus-leukemia effect expected
Inform 2nd transplant donor selection
Consider an alternative HLA mismatch
Guide relapse management
Select strategies according to the mechanism of immune escape