Understanding HLA Loss of Heterozygosity to Enhance Post-Hematopoietic Cell Transplant Therapeutic Decisions

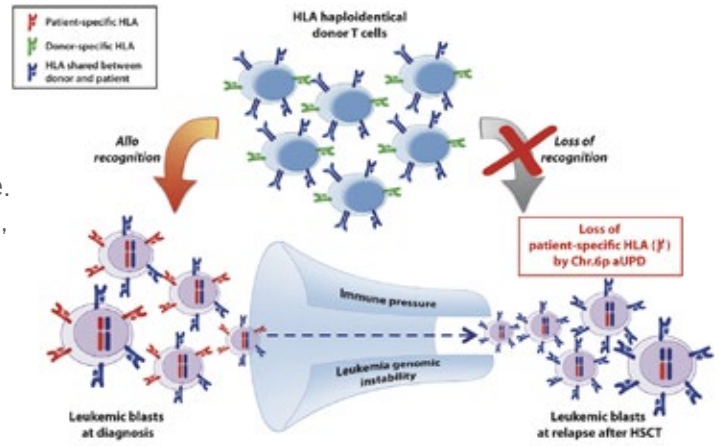
Versiti's Transplant Innovation Community is a quarterly webinar series that brings together individuals in the stem cell transplant and cellular therapy field to discuss various advances in transplantation and histocompatibility. The following recap is from a Transplant Innovation Community webinar presented by Jennifer J. Schiller, PhD, F(ACHI), Versiti's senior director of Histocompatibility and Immunogenetics, and Chad Hudson, MD, PhD, Hematologics, Inc.'s Medical Director - Clinical Trials and Research, on the role of HLA LOH in post-transplant relapse and how Versiti's unique assay can detect genomic loss in the HLA region. To access the full webinar, please visit versiti.org/lohwebinar.

What is HLA LOH?

HLA LOH, or loss of heterozygosity, refers to the genomic loss of heterozygosity in the HLA region, a phenomenon that can drive relapse after haploidentical and HLA mismatched hematopoietic cell transplantation (HCT)[1]. HLA LOH can occur in up to 30% of post-transplant relapses with haploidentical donors and between 5-10% of post-transplant relapses with unrelated donors[2].

Immune Evasion in Post-Transplant Relapse

One of the ways relapse post-transplant can occur is due to immune evasion mechanisms. With haploidentical donors, donor T cells can recognize unique HLA differences on the patients' cells, including the leukemic blast cells, which can produce an immune response. However, through the transplantation process, immune pressure and genomic instability in leukemia cells can lead to the loss of the patient-specific HLA antigens. This deletion of the mismatched HLA haplotype through an acquired somatic uniparental disomy (UPD) event hinders the donor T cells from recognizing the HLA on the surface of the blasts, enabling the leukemia to relapse.



Vago L, Toffalori C, Ciceri F, Fleischhauer K. Genomic loss of mismatched human leukocyte antigen and leukemia immune escape from haploidentical graft-versus-leukemia. Semin Oncol. 2012;39(6):707-715. doi:10.1053/j.seminoncol.2012.09.009

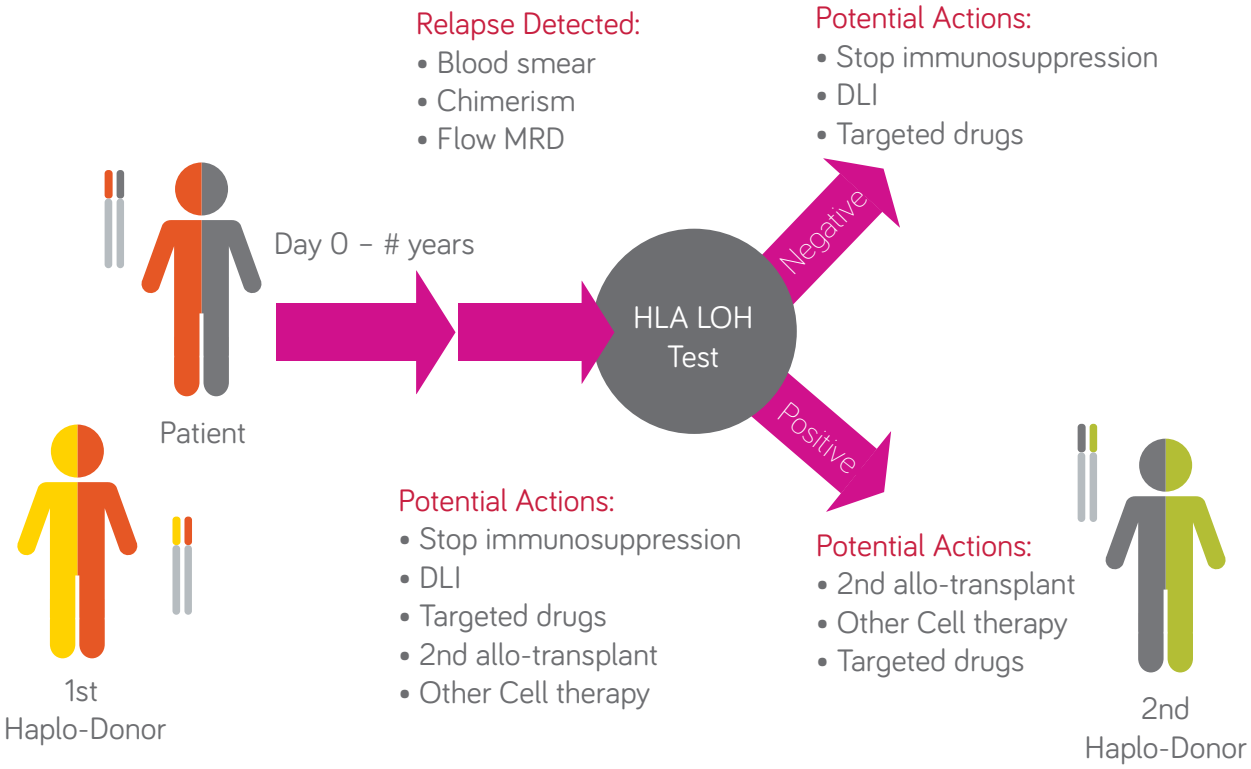
HLA LOH Clinical Assay Insight

The primary intent of developing the HLA LOH clinical diagnostic assay is to detect genomic loss in the HLA regions in post-transplant patients. This assay can identify allele-level HLA genomic loss in the context of donor and recipient genomes with as little as 1% blasts. The HLA LOH assay is a multi-analytic approach leveraging flow cytometry and genomic analysis. Working in collaboration with Hematologics, the assay also differentiates sub-clonal populations with unique or unknown immunophenotypes to provide a highly sensitive assay.

How Does Understanding HLA LOH Impact Therapeutic Decisions?

The HLA LOH assay enhances treatment decisions in known cases of relapse by characterizing a potential driver of relapse and providing detailed insights into the mechanism of relapse. It is not intended to replace existing assays for detecting relapse, which can be done through a variety of clinical testing methods such as blood smear, chimerism or flow MRD testing; rather, it provides additional insight into the mechanism of relapse. There are several potential treatment options to consider following a post-transplant relapse[3], and by determining the presence or absence of HLA LOH, physicians gain further understanding into the most effective therapeutic options for the patient. HLA LOH can impact treatment decisions post-relapse in the following ways:

- No Indication of HLA LOH: Potential actions include stopping immunosuppression, donor lymphocyte infusion (DLI) or using targeted drugs.
- HLA LOH Detected: Actions may involve considering a second allogenic transplant from a different donor or another cell therapy. The detailed LOH results can also help inform what to look for in the HLA typing of the second donor to increase the likelihood of an effective transplant.



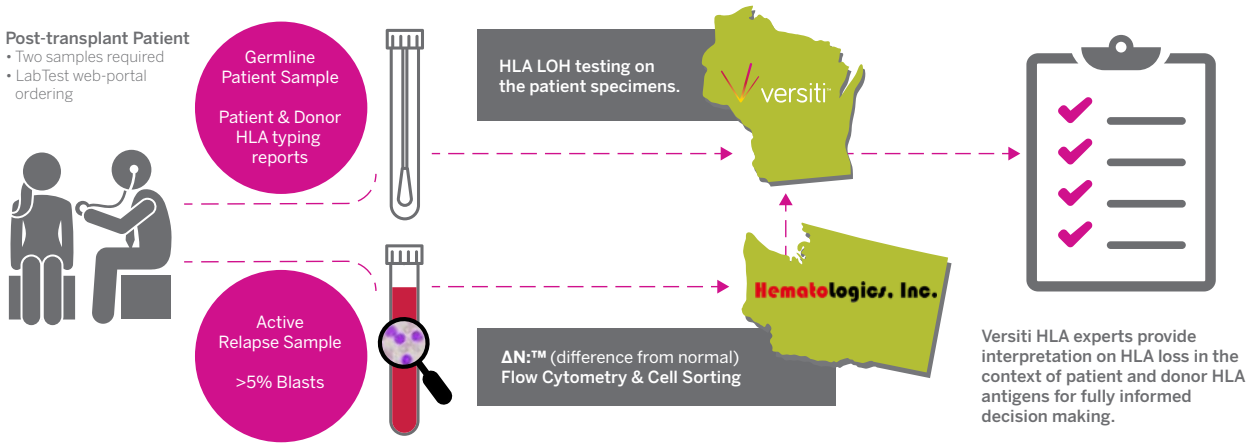
Methodology of the HLA LOH Assay

Versiti partnered with Hematologics to establish a robust, multi-analytical approach in detecting HLA LOH. Hematologics is a leader in residual disease testing with extensive expertise in flow cytometry. Versiti's genomic analysis methods combined with Hematologics flow cytometry provide accurate and detailed results and allow for comprehensive interpretations of the test result.

HLA LOH Evaluation Basics

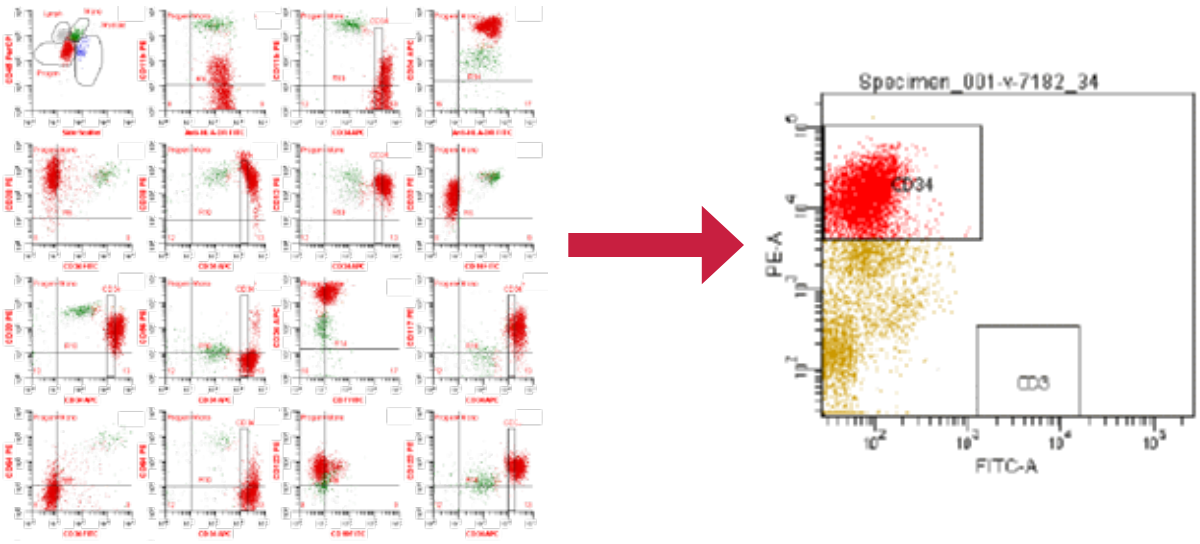
For accurate testing, an active relapse sample with >5% blast count (blood or bone marrow), a germline sample (buccal swab), and HLA typing reports from both patient and donor are required. The ideal sample collection should occur within a week of performing blast counts and before any treatment to ensure an adequate collection of blast cells.

The HLA LOH assay starts with a flow cytometric analysis of the relapse sample to sort and enrich for any relapsing blast cells, providing a clean DNA sample for genomic analysis. Then two different genomic analysis methods are performed to provide spatial and allele-level information across the major histocompatibility complex (MHC) region of chromosome 6. These analyses are compared to the pre-transplant patient and donor HLA typing profiles and provide specific detail on what HLA antigens are lost for fully informed, post-transplant care decision making.



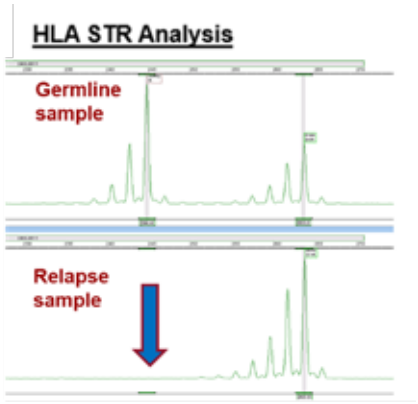
ΔN:™ (Difference from Normal) Flow Cytometry

Flow cytometry plays a crucial role in identifying abnormal populations or minimal residual disease (MRD) based on differences from normal hematopoietic bone marrow populations. Abnormal populations are identified through the expression of cell surface markers (CD antigens) and antigen expression intensity. ΔN:™ (Difference from Normal) Flow Cytometry by Hematologics focuses on the intensity of the expression and not cell frequency to determine residual disease. For a population to be considered abnormal, it must be a cluster of at least 40 cells or 0.02% of an adequate specimen, with abnormalities at least two standard deviations away from the normal populations. Once a cell population is determined as normal based on ΔN, the immunophenotype is utilized to sort the blast cells. The DNA from these sorted cells is then sent to Versiti for further genomic analysis.

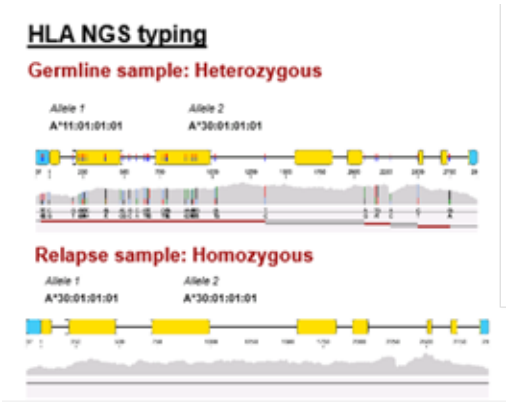


Genomic Analysis

Versiti performs a genomic loss analysis on the relapse sample and germline specimen using two complementary, orthogonal methods.



Each STR marker has two different alleles with a primary peak for each allele. In this example, the germline sample clearly has two peaks, where the relapse sample shows only one peak. This is a good indication that LOH has occurred in the region of this STR marker.



The germline sample in this example clearly shows heterozygosity with the polymorphic nucleotides of two different alleles visible within the gray bar. In the relapse sample, there is only one allele as evidenced by a lack of polymorphic nucleotides which is a clear indication that the locus has been deleted in the blast cell and LOH has occurred.

HLA STR Analysis

A spatial analysis is completed on a panel of nine highly polymorphic STR markers within the HLA region used to distinguish between patient and donor genomes. This method provides a sensitive indication of LOH, even with low levels of DNA recovered from a low percentage of blast cells early during relapse.

HLA NGS Typing

Performed alongside STR analysis, HLA NGS typing provides allele level assessment of the HLA loci to identify loss of specific, non-shared HLA antigens. A lack of heterozygous positions in the relapse sample indicates homozygosity and potential LOH.

In combination, these two assays provide a sensitive and spatially resolved assessment of LOH across the entire HLA genomic region. For mismatched, unrelated donor/recipient transplant pairs, the ability to detect a locus-specific HLA LOH event can be useful when the HLA typing may differ at only one or two HLA loci.

HLA LOH Case Studies

To date (April 2024), over 50 samples from post-HCT patients have been tested with a variety of diseases (AML, MPN, ALL). Valid samples ranged from <1% to 85% blasts or 800 to 250,000 cells. HLA LOH was detected in approximately 25% of tested cases. Below are three case highlights showing how the HLA LOH assay was utilized.

Morphologic Relapse

- Case: Pediatric AML with 35% blasts in peripheral blood and flow sort CD34+ yielded 150k cells.
- Findings: Aberrant myeloid progenitor cells with multiple abnormalities including decreased CD38 and heterogenous CD7 expression. LOH was apparent in all 9 STR markers and the HLA NGS typing the HLA loci were clearly homozygous in the relapse sample. Genomic analysis indicated loss of HLA haplotype A*11:01-B*55:01-DRB1*04:03-DPB1*06:01.
- Conclusion: Relapse sample had sufficient blasts that were well characterized and showed a clear indication of HLA LOH.

Low Blast Percentage

- Case: Adult MDS with less than 1% blasts in bone marrow and flow sort CD34+ yielded 0.8k cells.
- Findings: Despite low cell counts, multiple abnormalities were detectable via flow cytometry. LOH was confirmed with STR markers, though NGS typing was less informative due to low DNA yield from the available blast cells. Genomic analysis indicated loss of HLA haplotype A*33:01-B*14:02-DRB1*03:01-DPB1*04:02.
- Conclusion: The complementary, orthogonal molecular approaches were beneficial to confirm HLA LOH in low yield samples. Additionally, having the patient's and donor's pre-transplant typing made it possible to identify the haplotype that was lost.

Multiple Clones

- Case: Adult AML with 10-15% blasts in peripheral blood and flow sort two populations, CD117++/CD34 Hetero yielded 39k cells and CD117+/CD34+ yielded 53k cells.
- Findings: Multiple abnormal subclones identified; no HLA LOH detected.
- Conclusion: Ability to differentiate and analyze multiple subclones in one specimen and connect HLA loss to an immunophenotypic cell subtype.

Conclusion

The HLA LOH clinical assay offers critical insights into the mechanisms of relapse post-HCT. By leveraging high-sensitivity flow cytometry and HLA genomic expertise, this assay provides a detailed understanding of HLA LOH in the allogeneic transplant setting that can be used to guide effective treatment strategies during relapse.

For more information on Versiti's HLA Loss of Heterozygosity evaluation, visit versiti.org/loh

References:

1. Vago, Luca et al. "Loss of mismatched HLA in leukemia after stem-cell transplantation." *The New England journal of medicine* vol. 361,5 (2009): 478-88. doi:10.1056/NEJMoa0811036
2. Villalobos, Itzel Bustos et al. "Relapse of leukemia with loss of mismatched HLA resulting from uniparental disomy after haploidentical hematopoietic stem cell transplantation." *Blood* vol. 115,15 (2010): 3158-61. doi:10.1182/blood-2009-11-254284
3. Rovatti, Pier Edoardo et al. "Mechanisms of Leukemia Immune Evasion and Their Role in Relapse After Haploidentical Hematopoietic Cell Transplantation." *Frontiers in immunology* vol. 11 147. 25 Feb. 2020, doi:10.3389/fimmu.2020.00147

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