HLA Loss of Heterozygosity Evaluation

Versiti offers a unique approach for detecting and characterizing genomic loss of heterozygosity in the HLA region that can provide valuable information for patients with post-transplant relapse. Our assay starts with flow cytometric enrichment of relapsing cells followed by genomic analysis using two different methods to ensure accurate detection of genomic loss.

Acquired Loss of Heterozygosity (LOH) of the HLA region is observed in various cancers and has been shown to impact immunotherapy[1,2]. HLA LOH has been described as an underlying driver of disease relapse in haploidentical bone marrow transplants[3] where the relapsing cells have evolved to evade allorecognition by the donor cells. Post-transplant relapse is often poorly understood and the use of a test to characterize a potential driver of the relapse offers a new paradigm in diagnostic algorithms and could have treatment implications[4,5]. Loss of the non-shared HLA loci during relapse may indicate a loss of the graft leukemia effect and some treatments such as donor leukocyte infusion may not be effective.[6]

Versiti has created a comprehensive evaluation to detect genomic loss of heterozygosity in the HLA region specifically for transplant patients. We are collaborating with Hematologics, Inc to provide industry leading difference from normal ΔN:™ flow cytometry and cell sorting components of the evaluation. Additionally, we employ two methods for genomic analysis that provide spatial information across the MHC region as well as high resolution allelic detail across 9 classical HLA loci for complete genomic picture applicable in all transplant patient donor pairs. Other assays use limited markers within a few HLA loci and can be non-informative in 30-40% of transplants.[7] Finally, Versiti provides interpretation on HLA loss in the context of the patient and donor HLA antigens for fully informed decision making.

Indications for testing:

Intended for patients in the post-transplant relapse phase to evaluate the presence or absence of genomic loss of heterozygosity in the HLA region in blood and bone marrow cells.

Test Method

Flow cytometric analysis comparing normal to aberrant cells followed by cell sorting for a subset of markers to enrich the aberrant cells. Genomic DNA from the aberrant cells is analyzed for HLA loss of heterozygosity (LOH) using STR markers and next generation sequencing (NGS) of the HLA region located on chromosome 6p. Comparison of the germline sample and relapse sample is done at the genomic level for all informative markers.

Assay sensitivity and limitations:

Flow cytometric sorting is used to enrich the aberrant cell population prior to molecular analysis. The molecular assays are validated down to 1% sensitivity of markers in the HLA region. A quantitative assessment of LOH cannot be provided as the evaluation relies on the results of multiple diagnostic assays.

Reporting of results:

Full HLA LOH, Partial HLA LOH, No HLA LOH, or Indeterminate HLA LOH will be reported as well as the cell sort markers used to enrich the aberrant cells. Comments and interpretation may include details on the HLA regions or alleles lost relative to the nonshared HLA types between the patient and donor.

versiti™

Specimen requirements:

Order#2722 HLA Loss of Heterozygosity Evaluation requires fresh blood or bone marrow collection during active relapse, ideally with blast counts at 5% or greater.

- Peripheral blood: 5-10ml Na Heparin/Green top
- Bone marrow: 2-3ml Na Heparin/Green top
- Store sample ambient (DO NOT FREEZE) and ship same day. Must be received within 72 hours of collection.

Order# 2720 HLA LOH Patient Germline sample is also required to appropriately describe and reference the genomic loss of the aberrant cells. Pre-transplant samples of DNA can be accepted. NOTE: if your Transplant Center does not use Versiti for pre-transplant HLA typing, the HLA LOH Patient Germline sample is required.

- Preferred sample 4 buccal swabs, pre-transplant DNA is also acceptable.
- Store & ship ambient with an overnight carrier.
- HLA typing reports on the patient and donor are also required for the HLA LOH Evaluation.

SHIP

Shipping requirements:

Ship the package in compliance with your overnight carrier guidelines. Label with the appropriate address:

For HLA Loss of Heterozygosity Evaluation, order#2722 ship to:

Hematologics Inc
3161 Elliott Ave
Suite 200
Seattle, WA 98121

For HLA LOH Patient Germline, order#2720 ship to:
Versiti Client Services/
Histocompatibility Lab
638 N. 18th Street
Milwaukee, WI 53233
800-245-3117, ext. 6250

ORDER

Required forms:

HLA Loss of Heterozygosity Requisition

Turnaround time:

TAT: 8-12 calendar days based on time of sample receipt.

References:

1. Zhao J, et al. The prevalence of HLA LOH across 10 cancer types in Chinese patients. 2020, Journal of Clinical Oncology 38:15_suppl, 3124-3124
2. McGranahan, et al. Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution. 2017, Cell. 171, 1259–1271
3. Crucitti L, et al. Incidence, risk factors and clinical outcome of leukemia relapses with loss of the mismatched HLA after partially incompatible hematopoietic stem cell transplantation. 2015, Leukemia 29:1143-1152
4. Dholaria B, et al. Clinical applications of donor lymphocyte infusion from an HLA-haploidentical donor: consensus recommendations from the Acute Leukemia Working Party of the EBMT. 2020, Haematologica. 105(1):47-58
5. Imus PH et al. Major histocompatibility mismatch and donor choice for second allogeneic bone marrow transplant. 2017, Biology of Blood and Marrow Transplant. 23(11): 1887–1894.
6. Rovatti PE, et al. Mechanisms of Leukemia Immune Evasion and Their Role in Relapse After Haploidentical Hematopoietic Cell Transplantation. 2020, Frontiers in Immunology. 11: 147
7. Ahci M, et al. A new tool for rapid and reliable diagnosis of HLA loss relapses after HSCT. 2017. Blood. 130(10):1270-1273.

versiti™