A NOVEL MULTI-ANALYTIC CLINICAL DIAGNOSTIC ASSAY TO DETECT HLA LOSS OF HETEROZYGOSITY IN POST-HEMATOPOIETIC CELL TRANSPLANT RELAPSE PATIENTS

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INTRODUCTION

Genomic loss of heterozygosity in the HLA region (HLA LOH) can be an underlying driver of disease relapse in haploidentical and HLA mismatched hematopoietic cell transplantation (HCT) in which relapsing cells evade donor T-cell allorecognition by deleting non-shared HLA alleles through an acquired somatic uniparental disomy event. The presence of HLA LOH may indicate a loss of graft vs. leukemia effect, render treatments such as donor leukocyte infusion ineffective, and impact donor selection for subsequent allogeneic HCT.[1]

AIM

Develop a clinical diagnostic assay to detect HLA LOH post-HCT that can detect loss of genomic material against a background of multiple genomes. The approach needs to be effective early in relapse when the malignant cells are in low abundance and be able to identify sub-clonal populations with variable and/or unknown immunophenotypes. To address these issues, we have developed a comprehensive multi-analytic approach to detect HLA LOH that includes sensitive flow cytometric enrichment of leukemic cells followed by genomic analysis.

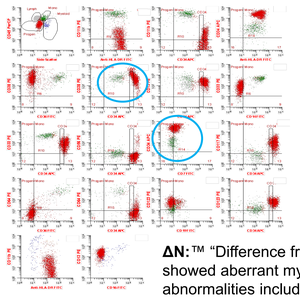
METHOD

1. Identify the aberrant cells by a “difference from normal” flow cytometry approach (**ΔN:**™)[3]
2. Perform flow cytometric sorting to enrich for aberrant cell clones
3. Perform comparative genomic analysis on the sorted aberrant cells and a germline specimen from the patient using two complementary molecular methods
4. Spatial analysis across the HLA region is assayed by nine short tandem repeat (STR) loci between classical HLA genes
5. Allelic level assessment of the HLA loci is provided by NGS based genotyping assay to identify loss of specific non-shared HLA antigens

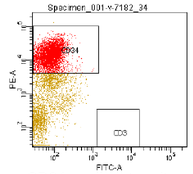
CASE #1 MORPHOLOGIC RELAPSE:

Pediatric AML with 35% blasts in peripheral blood
Flow sort CD34+ yielded 150K cells.
Genomic analysis indicated loss of HLA haplotype:
HLA-A*11:01-B*55:01-C*03:03-DRB1*04:03-DQB1*03:02-DPB1*06:01

ΔN:™ Immunophenotyping Flow Cytometry



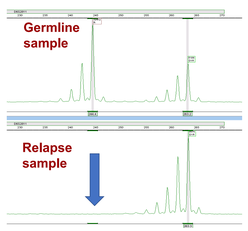
Cell Sorting by Flow Cytometry



CD34+ cell sort purity by flow cytometry plot

ΔN:™ “Difference from normal“ immunophenotyping showed aberrant myeloid progenitor cells with multiple abnormalities including decreased CD38 and heterogeneous CD7 expression

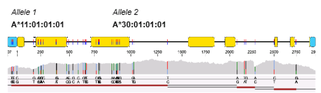
HLA STR Analysis



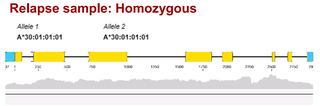
HLA STR results for one marker showing LOH in the relapse sample

HLA NGS typing

Germline sample: Heterozygous



Relapse sample: Homozygous

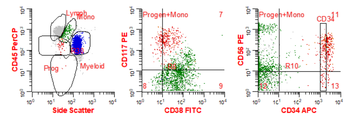


HLA NGS typing result for HLA-A showing LOH in the relapse sample

CASE #2 LOW BLAST %:

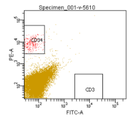
Adult MDS with <1% blasts in bone marrow
Flow sort CD34+ yielded 0.8K cells
Genomic analysis indicated loss of HLA haplotype:
HLA-A*33:01-B*14:02-C*08”02-DRB1*03:01-DPB1*04:02.

ΔN:™ Immunophenotyping Flow Cytometry



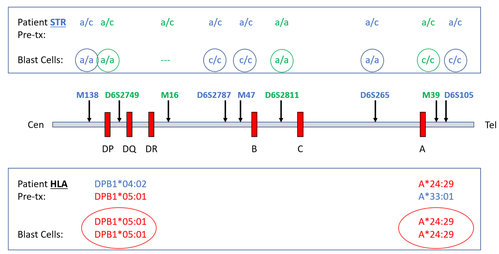
ΔN:™ immunophenotyping showed aberrant myeloid progenitor cells that expressed CD56 and decreased CD38

Cell Sorting by Flow Cytometry



CD34+ cell sort purity with very low cell yield (800 cells)

HLA STR & NGS Typing Analysis

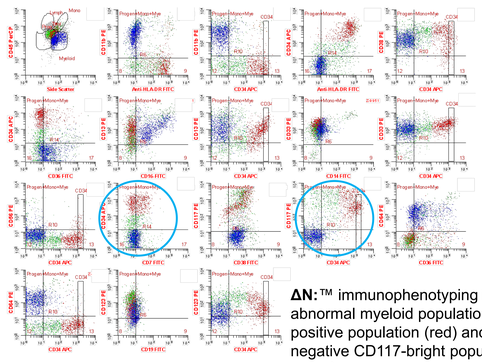


The low number of sorted blasts produced poor yield of DNA for the molecular assays. Sufficient HLA NGS reads were obtained at only the HLA-A and HLA-DPB1 loci, suggesting LOH across the HLA region. Despite low input DNA, the STR assay clearly showed LOH across the HLA region. These data show the benefit of combining two orthogonal molecular approaches to confirm HLA LOH in low yield samples.

CASE #3 MULTIPLE CLONES:

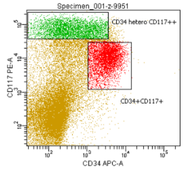
Adult AML with 10-15% blasts in peripheral blood.
Flow sorted two populations: CD117++/CD34 Hetero and CD117+/CD34+ with cell yields of 39K & 53K, respectively.
Genomic analysis indicated no loss of HLA

ΔN:™ Immunophenotyping Flow Cytometry



ΔN:™ immunophenotyping identified two abnormal myeloid populations, a CD34-positive population (red) and a CD34-negative CD117-bright population (light green).

Cell Sorting by Flow Cytometry



Two populations were recovered in the cell sort.
39K CD117++/CD34 Hetero
53K CD117+/CD34+

The major advantage of **ΔN:**™ flow cytometric analysis & sorting in the pre-analytical step is the potential to differentiate multiple subclones and connect HLA loss to an immunophenotypic cell subtype.

RESULTS

As of this printing, 18 samples from post-HCT relapse patients were tested with this assay and HLA LOH was detected in 3 cases (17%), consistent with published reports on the frequency of HLA LOH detected in allogeneic HCT patients.[1,2] Samples with a range of relapsed leukemic cells from <1% to 85% and cell sorting output from 800 to 250,000 cells all yielded valid results. Results were reported within an average of 7 days, allowing adequate time for integration into therapeutic decision making. Challenging and unusual patient samples are shown in case examples above.

CONCLUSIONS

In combination, the two assays provide a sensitive and spatially resolved assessment of LOH across the entire HLA genomic region, enabling molecular characterization of LOH events ranging from an entire HLA haplotype, partial HLA haplotype, or a single HLA locus. The ability to detect a locus-specific HLA LOH event could be useful in mismatched unrelated HCT donor/recipient pairs which may differ at only one or two HLA loci.

Initial results indicate this clinical diagnostic assay provides reliable and accurate detection of HLA LOH during relapse in post-HCT patients. Detection of HLA LOH using our multi-analytic diagnostic approach may also aid in optimizing therapeutic decisions to improve outcomes in allogeneic HCT patients.

REFERENCES

1 Vago L, et al. Loss of mismatched HLA in leukemia after stem-cell transplantation. *N Engl J Med.* 2009;361 (5):478-488

2 Dholaria B, et al. Clinical applications of donor lymphocyte infusion from an HLA-haploidentical donor: consensus recommendations from the Acute Leukemia Working Party of EBMT. 2020, *Haematologica.*2020; 105(1):47-58.

3 Loken, MR, Brodersen, LE, Wells, DA. *Monitoring AML Response Using “Difference from Normal” Flow Cytometry;* T.E. Druley, Minimal Residual Disease Testing, Current Innovations and Future Directions (pp 101-137). Springer International Publishing AG, 2019 Cham, Switzerland

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