Minimal residual disease (MRD) as a predictive biomarker/endpoint for novel drug development

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Introduction

Measurable Residual Disease or Minimal Residual Disease (MRD) is defined as the continual presence of cancer cells in patients post therapy that is below the threshold of detection by conventional morphologic or radiologic assessments. Monitoring patient status clinically as MRD negative or MRD positive has become the basis for predicting outcomes, remission or reoccurrence, as well as determining therapeutic options. The three primary methods to assess MRD status in patients include Flow Cytometry (FC), Polymerase Chain Reaction (PCR) and Next-Generation Sequencing (NGS). All three are highly quantitative measurements with a high degree of sensitivity to detect "new" circulating tumor cells or ctDNA that can potentially indicate disease recurrence before it happens. The choice of MRD methodology usually depends on the tumor type, liquid or solid. Flow Cytometry has been widely used to monitor MRD status in patients with hematologic malignancies. Specifically designed MRD panels have been used clinically with flow cytometry in CLL, MM, and B-cell Precursor ALL patients. In addition to its prognostic/diagnostic relevance, recent guidance by the FDA is now driving the use of MRD testing as a predictive biomarker/endpoint for novel cancer drug development. NeoGenomics Pharma Services has provided flow cytometry MRD expertise to multiple sponsors conducting clinical trials to evaluate the effectiveness of novel cancer therapy. At present, it is noted that a consensus panel for measuring minimal residual disease in Acute Myeloid Leukemia by flow cytometry has not yet been agreed upon by the scientific community at large due to the heterogeneity of the disease and its ability to mutate, and therefore, is not included in this presentation.

Clinical Value of Flow Cytometry MRD Testing

The objectives of treating cancer patients are to reduce/minimize the tumor burden and then hopefully go further and obtain a complete therapeutic response, resulting in total eradication or remission of the disease. However, post-treatment, if there are remaining (residual) cancer cells in the body, they can become active, proliferate, and relapse the disease in patients. MRD detection is an indication that the treatment was not completely effective or that the treatment was incomplete and needs to continue longer. MRD may also be an indication that all cancer cells did not respond to the given therapy or that malignant cells have become resistant to the anti-cancer therapies used. When a treated patient tests MRD negative (absence of residual cancer cells), this outcome is predictive of long remission periods and prolonged survival rates. MRD testing provides great clinical value by acting as a gauge to direct cancer treatments and to provide better patient care.

Unlike solid tumors, blood cancers such as multiple myeloma (MM), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) can be monitored by measuring the presence and frequency of cancerous cells in the peripheral blood or bone marrow. Well established MRD flow cytometry panels have been used universally and extensively to detect and measure the presence of malignant cells at low frequency. The expression of several cell surface markers can easily be used as criteria to discriminate neoplastic cells from normal cells. In addition, the expression level of "neoplastic" markers can be used to identify phenotypes associated with certain patient outcomes. Because of advantages such as high applicability, rapid turn-around time, intrinsic quality control, no need for baseline sample and cost-effectiveness, flow cytometry MRD data has been used prognostically for guiding treatment decisions in pediatric and adult leukemia patients as well as assigning patients into different MRD-based risk groups. There are several well-established MRD flow panels that have been used extensively by NeoGenomics to assist physicians and hospitals in diagnosis and treatment of cancer patients with hematologic malignancies.

Flow cytometry MRD testing offers comparable sensitivity to PCR and is nearly universally accepted for MRD detection requiring the presence of deviation from a normal pattern of lymphoid maturation or specific leukemia phenotypes. The laboratories that offer MRD assessments by flow cytometry require significant technical expertise from both the laboratory staff and interpreting pathologist in order to avoid erroneous results and provide consistency in the data. To achieve the sensitivity level of at least 1 malignant cell in 10,000 cells (0.01% or 10^{-4}), acquiring at least 500,000 events is necessary. To achieve the sensitivity level of at least 1 malignant cell in 100,000 cells (0.001% or 10^{-5}), acquiring at least 5,000,000 events is necessary. Acquisition of this many cells allows for statistically relevant, confident identification of clusters of at least fifty events at the lowest sensitivity threshold. It should be noted that a cluster containing as few as ten events with a clearly aberrant immunophenotype may be sufficient for confident identification. Identification of such small cell clusters requires a validated assay with low background, and high clinical expertise in hematopathology. Various factors, including non-specific antibody binding, improperly titered antibodies, inclusion of irrelevant events in blast analysis, and instrument maintenance and integrity can compromise the assay performance, and impact specificity and sensitivity.

The term biomarker is commonly understood as referring to a characteristic that is measured as an indicator of normal biologic processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Within this definition, MRD can be used and regarded as a potential biomarker.

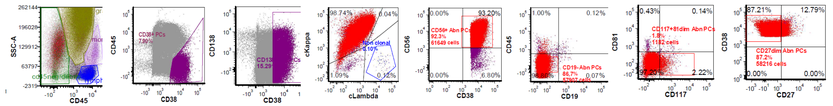
- **Diagnostic biomarker**: a biomarker used to detect or confirm the presence of a disease or conditions of interest or to identify individuals with a subtype of the disease.
- **Prognostic biomarker**: a biomarker used to identify likelihood of a clinical event, disease recurrence or progression in patients who have the disease or medical condition of interest. A prognostic biomarker informs about the natural history of the disease in that particular patient in the absence of a therapeutic intervention.
- **Predictive biomarker**: a biomarker used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a drug product.
- **Efficacy-response biomarker**: a biomarker that is used to show that a response has occurred in an individual who has been exposed to a drug product.
- **Monitoring biomarker**: a biomarker measured serially and used to detect a change in the degree or extent of the disease.

Flow Cytometry MRD Panels

Multiple Myeloma (MM) MRD panel

CD45, CD19, CD20, CD27, CD38, CD45, CD56, CD81, CD117, CD138, cKappa, and cLambda

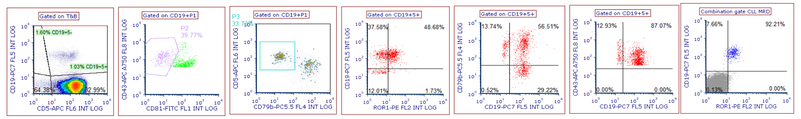
- This MM MRD panel has been shown to detect MRD in patients with previously diagnosed and treated multiple myeloma. The limit of detection is 0.001%.



Chronic Lymphocytic Leukemia (CLL) MRD Panel

CD45, CD3, CD5, CD19, CD20, CD22 (or ROR1), CD43, CD79b, CD81

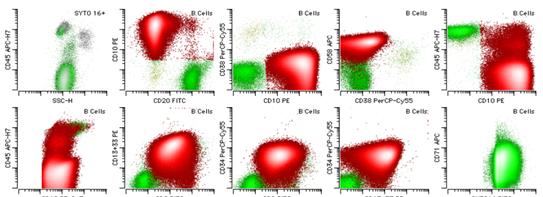
- This CLL MRD panel follows the strategy developed by the European Research Initiative in CLL (ERIC) and can detect MRD at the 0.01% level. This evaluation has become increasingly important as specific CLL treatments improve. Detection of MRD above 0.01% is reported to be an independent predictor of progression-free survival and overall survival in CLL patients treated with chemoimmunotherapy. The prognostic value of achieving MRD-negative status with other CLL therapies is under investigation in clinical trials.



B-Cell/B-precursor Acute Lymphoblastic Leukemia (B-ALL) MRD Panel

CD45, CD3, CD9, CD10, CD19, CD20, CD34, CD38, CD58, CD13/CD33, CD71, Syto16

- This B-ALL MRD panel follows the strategy developed by the Children's Oncology Group (COG) and can detect MRD at the 0.01% level. The B-ALL MRD is highly predictive of relapse in patients treated for acute lymphoblastic leukemia



Patient Progression Data

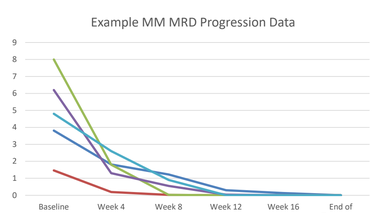


Chart 1: The flow results shown in Chart 1 shows clinical trial data utilizing MRD assessment for Multiple Myeloma at multiple time points following initiation of clinical trial treatment with a prospective monoclonal therapy within refractory/relapse patients.

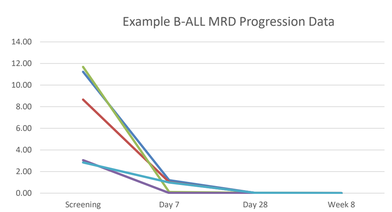
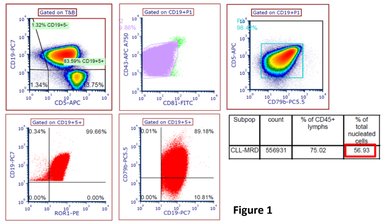


Chart 2: The flow results shown in Chart 2 shows clinical trial data utilizing MRD assessment for B-Cell/B-precursor Acute Lymphoblastic Leukemia at multiple time points following initiation of clinical trial treatment with a prospective autologous CAR-T therapy within refractory/relapse patients.

Example Data Analysis

CLL MRD Patient Progression

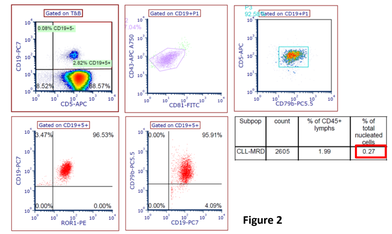
Timepoint 1 (Baseline)



Subpop	count	% of CD45+ lymphs	% of total nucleated cells
CLL-MRD	556931	75.02	56.93

Figure 1

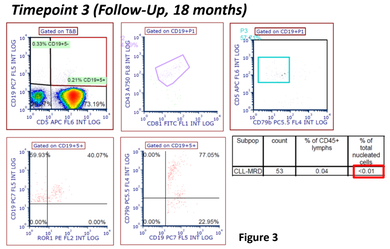
Timepoint 2 (6 months)



Subpop	count	% of CD45+ lymphs	% of total nucleated cells
CLL-MRD	2605	1.99	0.27

Figure 2

Timepoint 3 (Follow-Up, 18 months)



Subpop	count	% of CD45+ lymphs	% of total nucleated cells
CLL-MRD	53	0.04	<0.01

Figure 3

Sequential MRD Assessment CLL	
Time Point	**%MRD**
Baseline	56.93%
~ 6 months	0.27%
~18 months	<0.01%

Figures 1-3 The flow results shown in Figures 1, 2 and 3 illustrate the value of MRD. The data provides a precise indication of therapeutic efficacy over the course of treatment. Sequential testing of peripheral blood samples over time indicates that % MRD levels fell dramatically from baseline (56.93) to iCR at 6 months (0.27), and CR at around 18 months (<0.01). Clearly this is an example of how MRD can serve as a "biomarker" to evaluate a patient's response to novel drug therapy in clinical trials. In addition, these data can be used to determine treatment processes, such as timing and dosage, as well as the use of MRD to identify responder and non-responder patient populations.

Conclusions

The evolution of regulatory science to allow innovative approaches, such as MRD, to the development of new therapeutic treatments for hematologic malignancies could expedite approval of drugs that could potentially improve patient outcomes. Incorporation of MRD into clinical trial evaluation protocols in which sequential analysis can be performed may determine the efficacy of novel investigational drugs earlier by acting as a surrogate biomarker of patient outcome and endpoint in clinical trials, compared with current traditional approaches. Since MRD measures tumor burden at levels that are undetectable through conventional lab techniques, it can also potentially act as a clinical and regulatory endpoint, which supports its use in future clinical trials as well as for clinical decision making. NeoGenomics provides our clients with an unparalleled level of expertise, service, flexibility, and scalability. The data shown and generated by NeoGenomics displays the quality and utility of MRD analysis in the context of patient monitoring.

Ongoing and future clinical trials will continue to evaluate the definition and the role of MRD in treatment decision-making. On the one hand, the achievement of an MRD-negative status does not necessarily mean that treatment should be stopped, or that a new therapy can cure the disease. With the limitations of all MRD testing, it means that we can't be sure that the disease is eradicated even in MRD-negative cases. On the other hand, an MRD-positive status after treatment brings into question whether it is necessary to change treatment or dosage, improving the depth of response. However, before developing response-adjusted treatment strategies based on MRD status, either intensifying or changing treatment for MRD-positive patients or de-escalating treatment for MRD-negative patients, we need to determine if sustained MRD negativity should be viewed as the goal of any treatment. Continued use of MRD as a biomarker in clinical trials will provide information that will help define the most appropriate time point for its evaluation as a clinical endpoint.

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