Measurement of Circulating Tumor DNA to Guide Management of Patients with Lymphoma

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Abstract In recent years, advances have been made in methods to assess response to therapy in lymphoma. Ideally, response assessment tools should be highly sensitive and specific for identifying a disease, should carry a minimal risk of harm to the patient, and should provide reproducible results. Traditional surveillance methods have included clinical assessment and, in many cases, routine surveillance imaging. Minimal residual disease (MRD) refers to the detection of disease level below that of these traditional surveillance methods. Either circulating tumor cells or their nucleic acid fragments released from necrotic/apoptotic cells can be measured in circulating peripheral blood, referred to as circulating tumor DNA (ctDNA). ctDNA can be detected with allele-specific polymerase chain reaction (ASO-PCR) or with next-generation sequencing (NGS) techniques. The use of ctDNA as a monitoring strategy in lymphoma can aid in assessment of disease burden, as well as prognostication, customization of therapy ("risk-adapted" strategies), monitoring for relapse, and consideration of early intervention ("preemptive" strategies), while reducing radiation exposure from surveillance imaging modalities that are presently used. In this review, we discuss the current state of the art in ctDNA measurement, as well as the clinical data supporting its potential utility in the management of lymphoma patients.

Introduction

Lymphomas are a heterogeneous group of neoplasms with widely varying clinical and biological features. However, the strategies used by clinicians to assess response to treatment and to monitor for relapse are relatively homogeneous across the lymphoma subtypes. These strategies include assessment of symptoms, physical examination, basic laboratory studies, imaging (computed tomography [CT], ^{18}F-fluorodeoxyglucose–positron emission tomography/computed tomography [^{18}F-FDG PET/CT]), and tissue sampling (bone marrow, blood, lymph nodes). Minimal residual disease (MRD) refers to the presence of a small number of cancerous cells that can be detected through the use of cytometric or molecular

techniques but not by routine surveillance methods. The most commonly used methods for detection of MRD are multiparameter flow cytometry (MFC), allele-specific oligonucleotide polymerase chain reaction (ASO-PCR), and next-generation sequencing (NGS). The latter 2 approaches rely on detection of circulating tumor DNA (ctDNA), typically in the peripheral blood and less commonly in the bone marrow. MRD has been evaluated in a variety of hematologic malignancies, including lymphoma. Potential applications of MRD include prognostication at various times, including at diagnosis, during treatment, and at the completion of therapy. In principle, this information could be used to escalate or de-escalate treatment or to dictate other interventions at these points ("risk-adapted" strategies). MRD can also be used in monitoring for relapse, with the goal that early identification of relapse will translate to early intervention that might delay or prevent overt clinical relapse (a "preemptive" strategy). This article reviews the current data on measurement of ctDNA in the management of patients with B-cell non-Hodgkin lymphomas.

Present Monitoring Strategies

Traditional methods of surveillance in patients who have received treatment for lymphoma include clinical and radiographic surveillance. Clinical surveillance consists of clinic visits, assessment of symptoms, physical examination, and routine laboratory studies. If the clinical assessment raises a concern for relapse, radiographic studies are ordered. Radiographic surveillance (or routine surveillance imaging) refers to CT or ^{18}F-FDG PET/CT imaging obtained on a planned schedule, regardless of whether clinical evidence of relapse exists. To date, no study has conclusively shown improved outcomes in lymphoma patients undergoing radiographic and clinical surveillance vs clinical surveillance alone. In fact, across several lymphoma subtypes, numerous retrospective studies have shown that radiographic surveillance is associated with no improvement in survival, increased cost, increased exposure to radiation, and numerous false-positive results.[1-6] Some studies have suggested that an increased risk of future malignancies could be attributed to radiation from CT scans,[7,8] although these data are largely theoretical. Given the risks, cost ineffectiveness, false-positive results, and lack of clear impact on outcome, radiographic surveillance is not the ideal monitoring modality in lymphoma. Despite these shortcomings, radiographic surveillance after lymphoma treatment is still frequently utilized in clinical practice. In addition, extensive surveillance imaging is often required for patients participating in clinical trials.

Minimal Residual Disease: Methods of Detection

MRD analysis has the potential to detect residual disease with greater sensitivity and specificity than imaging, but without radiation exposure, and potentially at a lower cost.[9] Techniques for MRD analysis include MFC, ASO-PCR, and NGS.

MFC is one method of detection that is well established in the diagnosis and monitoring of hematologic malignancies. MFC requires the pattern of cell surface markers to be both unique to the disease in question and found in sufficient levels.[9] The detection limit of modern MFC is generally accepted to be in the range of 1×10^{-4}.[10]

Measuring MRD from peripheral blood and bone marrow samples using circulating tumor DNA (ctDNA) is an active area of research in lymphoma. ctDNA refers collectively to DNA within intact circulating tumor cells, as well as fragments of circulating tumor DNA in the bloodstream. Cell-free DNA (cfDNA) is composed of both ctDNA and DNA released from nonmalignant cells of the body (Figure). In general, cancer patients have been shown to have higher levels of circulating cfDNA than healthy controls.[11-13] The release of cancerous cfDNA is thought to result from proliferation, apoptosis, and necrosis of malignant cells.[14-16] Macrophages that phagocytose dead cells can also release nucleic acid debris into the circulation. In addition, nucleic acid fragments can be actively released.[17,18] The length of cfDNA is often in multiples of 180 base pairs, suggesting that apoptosis likely contributes to the majority of cfDNA in circulation.[15,19] The cfDNA is efficiently cleared from the circulation by liver, kidney, and blood nucleases, with half-lives ranging from 15 minutes to a few hours.[20] However, the continuous release of cfDNA into the circulation allows for detection of ctDNA in various malignancies, with reports of detection dating back to as early as 1977.[11]

Depending on the ctDNA measurement technique, ctDNA, cfDNA, or both may be measured. ASO-PCR frequently relies on detection of circulating tumor cells and hence is more useful in lymphomas with blood/bone marrow involvement. NGS can detect both cellular and cell-free DNA. However, in the case of lymphomas, especially diffuse large B-cell lymphoma (DLBCL), the cfDNA compartment is significantly larger since DLBCL generally has minimal intact circulating lymphoma cells. NGS assays rely on measuring cfDNA in peripheral blood samples. Specialized tubes such as Streck tubes stabilize the circulating cells and prevent lysis, in addition to inhibiting the activity of circulating nucleases, thereby resulting in reliable detection of ctDNA.[21] In ASO-PCR, primers specific for genetic regions of interest or allele-specific oligonucleotides are designed to

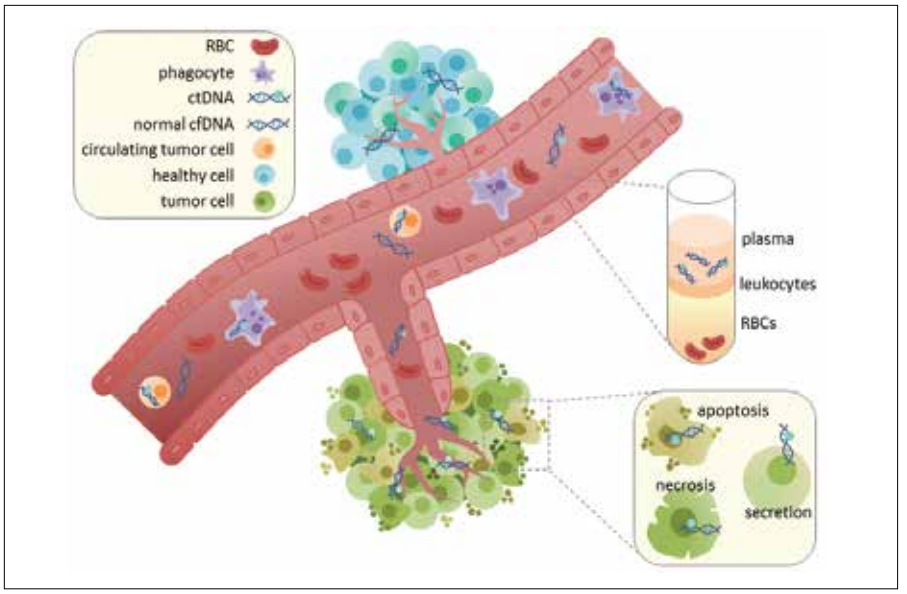

Figure. Circulating tumor DNA (ctDNA) is found in serum and plasma fractions from blood. The mechanism of ctDNA release is unknown, although apoptosis, necrosis, and active secretion from tumor cells have been hypothesized.

cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; RBC, red blood cell.

target the clonal immunoglobulin heavy chain variable region (*IGHV*), the clonal immunoglobulin light chain variable region (*IGLV*), immunoglobulin junctional regions, *IGH-CCND1*, or other translocations known to be present in a particular patient, followed by amplification with quantitative PCR.[22-24] Detection limits of 1 cell among 100,000 white blood cells can be reproducibly reached.[25,26] In mantle cell lymphoma (MCL), ASO-PCR has been validated as a dynamic marker of treatment response and a predictor of treatment outcomes.[24,27] However, in a significant number of patients, junctional regions are not sufficiently sensitive.[24,28] In the analysis of the MCL Elderly and MCL Younger cohorts, 14% of patients failed to be reliably quantified, and could not have their MRD status monitored by real-time quantitative PCR (RQ-PCR).[27,29] Moreover, consensus primers can detect the clonal *IGH* rearrangement in only a limited number of patients, and patient-specific primers are required in most cases, making the process resource- and labor-intensive. In addition, ASO-PCR as studied in MCL has relied on detection of circulating tumor cells. In other lymphomas such as DLBCL and marginal zone lymphoma, in which circulating tumor cells are limited and most patients lack a targetable chromosomal rearrangement, ASO-PCR has very limited utility.[30]

Some of these limitations can be overcome by the use of newer NGS-based methods. Two such methods have been evaluated in lymphoma: immunoglobulin high-throughput sequencing (Ig-HTS) and hybrid capture–based NGS. Ig-HTS involves identification and tracking of the unique immunoglobulin sequence from the malignant B cell. Hybrid capture–based NGS relies on identification and tracking of somatic genetic alterations associated with the malignancy. These methods have been studied in a variety of lymphoma subtypes, including DLBCL, MCL, and Hodgkin lymphoma. As opposed to ASO-PCR, the NGS-based methods do not need patient-specific reagents, are more widely applicable, and have lower limits of detection. In addition, the currently available NGS-based ctDNA methods rely on detection of cfDNA, as opposed to circulating tumor cells.

Immunoglobulin High-Throughput Sequencing

Ig-HTS relies on identifying one or more clonotypes in the baseline tumor tissue by using universal primers that target the immunoglobulin genes, followed by NGS of the targeted region. A clone is considered lymphomatous if it exists with a frequency greater than 5% in the tumor tissue. If baseline tumor tissue is not available, the clonotypes can also be identified using frozen cells, blood, or bone marrow clots, but the clonotype detection yield is significantly lower (37% for DLBCL and 67% for MCL).[22,31] The clonotype detection rate also differs with the type of tissue, with fresh tumor tissue providing a higher yield than formalin-fixed paraffin-embedded (FFPE) specimens. One study reported a calibration rate of 93% for fresh tumor tissue and 53% for FFPE in DLBCL.[32] However, with improving technology, an increasing number of clonotypes are being identified from FFPE specimens. In more recent reports, 84% of patients with DLBCL and 100% of patients with MCL had a calibrating rearrangement identified from baseline FFPE specimens.[22,31] The amount of baseline input DNA significantly affects the clonotype identification rate, and higher calibration rates are achieved with larger amounts of input DNA.[31,32]

In an early study, Ig-HTS was shown to be at least as sensitive and specific as ASO-PCR, with excellent concordance in most cases of acute lymphoblastic leukemia, MCL, and multiple myeloma.[33] Among 55 patients, 45 clonotypes were identified by RQ-PCR and 49 by Ig-HTS. Studies using Ig-HTS to measure MRD have found that MRD correlates well with radiographic disease burden.[32]

Another characteristic of Ig-HTS is its ability to detect clonotypes that are similar but not identical to the most frequent clones. Clonal heterogeneity and clonal evolution can be tracked by comparing immunoglobulin somatic mutations at diagnosis and upon follow-up, as demonstrated in follicular lymphoma and DLBCL.[31,34] However, this ability is limited by the fact that it does not account for heterogeneity in the other common areas of genomic alterations in lymphoma patients. As a result, clonal evolution from newly arising oncogenic "driver" mutations or mutations conferring resistance to certain therapeutic agents are not detected with Ig-HTS because only the immunoglobulin sequences are being analyzed.

Hybrid capture–based NGS involves identifying and tracking genetic alterations that are present in the tumor and detectable in peripheral blood.[35-38] With hybrid capture–based NGS, a library of somatic genetic alterations commonly observed in a particular tumor type is generated. These alterations include single-nucleotide variants (SNVs), insertions/deletions, and breakpoints involving oncogenes, tumor suppressor genes, and genes that play a role in canonical fusions (eg, *BCL2, BCL6, MYC,* and *IGH*), as well as the IGHV, IGLV, or junctional regions. After shearing patient DNA in a plasma sample, hybridization probes directed specifically at these regions of interest are added. DNA that is unbound to these hybridization probes is removed using magnetic methods. DNA that is captured by the probes is then amplified using PCR, and sequenced with NGS.[38] This identifies various tumor-associated genetic alterations unique to that patient's lymphoma, which can then be monitored in real time. Because the same library of genetic alterations can be utilized for a particular disease type, clonotype assessment of baseline tumor tissue is not required, in contrast with Ig-HTS.[37] Using this technique, detectable ctDNA could be identified in nearly all DLBCL patients with pretreatment plasma samples.[36,37] Furthermore, this technique obtains a genetic snapshot of the ctDNA at regular time intervals and can provide useful information about clonal heterogeneity and clonal evolution. Tumor biopsies are subject to sampling bias, whereas genotype detection using ctDNA can provide a more comprehensive overview of the collective mutational landscape of tumors present throughout the body. New genetic alterations and changes in allele frequencies of known mutations can be tracked, and have been shown to correlate well with the genetic makeup of the tissue biopsies at the corresponding times, thus serving as liquid biopsies.[35-37,39]

Both NGS-based techniques can reach lower detection thresholds than RQ-PCR, where the limit of detection is on the order of 1×10^{-5} (1 cell among 100,000 white blood cells). Ig-HTS has been shown to detect 1 haploid genome equivalent per 1,000,000 genome equivalents of input DNA, and hybrid capture–based NGS has been shown to detect a mutant allele fraction of 0.02% (1 in 5000) in peripheral blood.[22,37] However, the real-world sensitivity may be limited by the amount of total cfDNA available in plasma samples (larger sample volume results in higher input DNA and increases sensitivity). Utilizing Ig-HTS, ctDNA was detectable in 92% of pretreatment DLBCL samples and 98% of pretreatment MCL samples from patients in whom a calibrating rearrangement could be identified from baseline tumor tissue.[31,40] In the case of hybrid capture–based NGS methods, outcomes may also be affected by low variant allele frequency (VAF) of certain mutations. Somatic mutations with allelic abundance of less than 20% may not be accurately identified, as shown in one study of DLBCL patients.[35] Digital error suppression combined with molecular barcoding strategy can overcome this limitation, enabling recovery of ctDNA-harboring mutations with very low VAF.[41] In a more recent study using hybrid capture–based NGS, ctDNA was detectable in 98% (212/217) of pretreatment plasma samples in patients with DLBCL.[36]

ctDNA can be detected in all lymphoma subtypes, although levels vary somewhat.[42] Even in classical Hodgkin lymphoma, in which the malignant (Reed-Sternberg) cells are relatively rare in tissue biopsies, ctDNA analysis is feasible.[39,43] Although T-cell lymphomas can also be analyzed using a similar NGS strategy on the variable regions of the T-cell receptor (in a manner analogous to Ig-HTS) or by hybrid capture–based NGS, for the remainder of the review we will focus on application of ctDNA in B-cell non-Hodgkin lymphoma patients.

Potential Applications of ctDNA MRD in B-Cell Lymphoma Patients

Prognostic Value at Diagnosis

Quantitation of ctDNA levels may be used to aid in prognostication at diagnosis. ctDNA can be detected in most pretreatment specimens.[36,40,42,44] Some studies have shown that an increased level of ctDNA correlates with tumor burden, higher-stage disease, and lactate dehydrogenase, as well as inferior event-free survival (EFS) and overall survival (OS).[31,36,40,44-46] For example, Roschewski and colleagues demonstrated that median concentrations of

circulating tumor DNA were 416 (range, 0 to 2.4×10^4) lymphoma molecules per 10^6 diploid genomes for patients with low-risk (0-1) International Prognostic Index (IPI) scores, 5095 (0 to 1×10^6) lymphoma molecules per diploid genomes for those with intermediate-risk (2-3) IPI scores, and 7226 (3 to 1.6×10^5) lymphoma molecules per 10^6 diploid genomes for patients with high-risk IPI scores.[31] Kurtz and colleagues showed an association of ctDNA levels (as measured by hybrid capture–based NGS) with EFS in both frontline and salvage settings for patients with DLBCL, with lower ctDNA levels being associated with improved EFS.[36]

Two studies have evaluated the correlation between high pretreatment ctDNA levels and greater baseline disease burden. Kurtz and colleagues showed a significant linear correlation between high pretreatment ctDNA levels as measured by hybrid capture–based NGS and larger total metabolic tumor volume (TMTV) in DLBCL.[45] A similar linear association between pretreatment quantitative ctDNA levels as measured by Ig-HTS and tumor TMTV was seen in MCL patients.[40] In this latter study, however, although the baseline ctDNA level correlated with TMTV, it was not predictive of PFS or OS. More research is therefore needed to determine if quantitation of ctDNA at the time of diagnosis will ultimately be clinically useful and allow for risk stratification of lymphoma patients. It is more likely that the dynamic changes in ctDNA during or after completion of treatment will provide more powerful and clinically relevant prognostic information.

Assessing Response to Therapy

ctDNA can be used during therapy to track response and to potentially detect the emergence of mutations that confer drug resistance. Owing to ease of sampling, ctDNA can be obtained serially for quantitation over time to track clonal evolution. ctDNA levels change rapidly in response to treatment, with multiple log decreases noted within 1 week of treatment.[36] ctDNA can therefore provide an early readout of sensitivity to therapy, and decreases in level of ctDNA as early as after 1 cycle of treatment with chemotherapy or targeted agents have been shown to be associated with survival in both DLBCL and MCL.[35,36,40] Complete molecular response (ie, achieving MRD negativity) also has been associated with improved duration of clinical response.[27]

Kinetics and timing of MRD negativity also relate to outcomes. Two studies presented at the 2018 American Society of Hematology Annual Meeting found that patients with MCL who achieve MRD negativity earlier in therapy had improved outcomes, such as progression-free survival (PFS).[40,46] In one study, clearance of ctDNA after cycle 1 of induction was strongly associated with longer median PFS (76 vs 20 months; P=.0037) and a trend toward superior 4-year OS (92% vs 73%; P=.23).[40] Similar findings have also been reported with DLBCL.[36,44] One study in DLBCL patients evaluated ctDNA using hybrid capture–based NGS, after 1 cycle and after 2 cycles of first-line therapy or salvage therapy. Early molecular response (EMR) was defined as a 2-log decrease in ctDNA after 1 cycle, whereas major molecular response (MMR) was defined as a 2.5-log decrease in ctDNA after 2 cycles. Patients who achieved EMR and MMR during first-line therapy were found to have a greater EFS at 24 months. Among patients undergoing treatment for relapsed or refractory DLBCL, EMR (but not MMR) was associated with improved EFS. Additionally, molecular response was able to predict EFS and OS, independent of the IPI score or interim PET status.[36] Additional studies have shown that persistence of lymphoma-associated ctDNA at certain points is associated with a poor response to therapy.[47,48] In some cases, patients who ultimately developed treatment resistance showed new mutations arising (clonal evolution) that were detectable in ctDNA prior to overt clinical progression.[35,49,50] This may allow clinicians to identify patterns of resistance earlier, and ultimately to change treatment earlier.

Evaluating the efficacy of immunotherapy can be difficult because early treatment can be associated with a tumor inflammatory (or "flare") reaction, sometimes referred to as pseudoprogression. ctDNA can potentially provide more accurate information in measuring tumor response than imaging-based response criteria alone.[51] Presently, the most widely used response criteria in NHL is the Lugano classification,[52] which is based on the 5-point Deauville scale generated using ^{18}F-FDG PET/CT imaging. ^{18}F-FDG PET/CT has a very high negative predictive value at the end of treatment. However, some patients with a negative ^{18}F-FDG PET/CT at the end of treatment will relapse. Furthermore, the role of interim ^{18}F-FDG PET/CT scans in predicting the outcomes in DLBCL is not well defined because inflammation and tumor necrosis may lead to false-positive interpretation of images. Moreover, intensification of treatment based on interim ^{18}F-FDG PET/CT scans has not yet been shown to improve outcomes in most lymphoma subtypes. Response criteria incorporating both ^{18}F-FDG PET/CT and ctDNA should be evaluated to determine if this approach could provide a more robust assessment of clinical outcomes during therapy, as well as at the end of therapy.

Frontline therapies may induce long-term remission in indolent lymphoma, and following large cohorts of patients over long periods of time to assess survival in clinical trials can be technically and financially challenging. ctDNA may provide a much-needed surrogate endpoint

in place of PFS for such studies, and could potentially aid in expedited development of novel therapies in this space.

Application During Surveillance

Several studies have shown the prognostic value of MRD after completion of first-line therapy. In the Nordic MCL2 trial (2nd Nordic Mantle Cell Lymphoma Trial), 160 MCL patients younger than 66 years underwent first-line intensive induction therapy with dose-intensified rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (maxi–R-CHOP), alternating with rituximab plus high-dose cytarabine. Those who responded then received high-dose chemotherapy with carmustine, etoposide, cytarabine, and melphalan or cyclophosphamide (BEAM or BEAC) followed by autologous hematopoietic cell transplant (auto-HCT). For patients in whom a molecular marker of disease was identified, serial MRD assessment with ASO-PCR was performed for either the clonal IgH rearrangement or translocation t(11;14). Those who transitioned from MRD-negative to MRD-positive without clinical relapse were offered preemptive rituximab in an attempt to prevent clinical relapse (see section on Pre-Emptive Therapy below). In terms of prognostic value during surveillance, the patients who became MRD-positive during the first year of surveillance had a poor outcome, with median PFS of only 1.5 years.[53] Four other studies in MCL have shown that negative MRD status at the end of induction/pre–auto-HCT, or at a 1-year or 2-year landmark, is highly predictive of subsequent sustained remission duration.[25,27,54,55] This has been observed in patients undergoing intensive or nonintensive induction.

In DLBCL, multiple studies have demonstrated the utility of ctDNA in surveillance. In a prospective study utilizing Ig-HTS MRD in 75 patients with DLBCL, molecular relapse (ie, becoming MRD-positive) was noted to precede ^{18}F-FDG PET/CT detection of relapse, with a median lead time of 88 days (range, 14-162 days).[32] ctDNA was measured during surveillance for 25 patients, of whom 5 eventually relapsed. Three of the 5 patients (60%) had ctDNA detectable in the plasma before their overt relapse. The remaining 2 patients had detectable ctDNA in the plasma at the time of relapse. A separate retrospective study evaluated a cohort of patients with DLBCL who had been treated in 1 of 3 protocols using etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (EPOCH) with or without rituximab. Surveillance ctDNA samples were collected at predetermined points in 107 patients who achieved a complete remission (CR).[31] Of the 17 patients whose disease relapsed, ctDNA was detectable in serum prior to clinical relapse in 15 (sensitivity, 88.2%), with a median lead time of 3.5 months prior to clinical/radiographic relapse. Moreover, patients had a reduced burden of disease when recurrence was identified with ctDNA vs imaging. Only 2 of 90 patients who did not relapse had a nonreproducible positive ctDNA assay (specificity, 97.8%).[31] In a smaller study utilizing hybrid capture–based NGS as a monitoring tool, 30 DLBCL patients who underwent first-line treatment with R-CHOP were profiled and monitored serially for ctDNA, with 25 of 30 patients achieving a PET-negative CR. Among patients who were primarily refractory to R-CHOP, persistent ctDNA remained detectable. Among the 2 patients who achieved CR but ultimately relapsed, one patient had ctDNA that was detectable 1 month prior to clinical relapse. In addition, new mutations (clonal evolution) arose in some patients during therapy.[35]

The value of surveillance ctDNA was also evaluated in a study of 88 lymphoma patients who underwent allogeneic HCT. In this study, 19 patients ultimately developed clinical relapse or progression. Molecular relapse was identified (using Ig-HTS) on average 3.7 months prior to overt relapse/progression in 16 of 19 (84%) patients. In one additional patient, ctDNA became detectable at the time of relapse.[56]

The value of early detection still needs to be evaluated prospectively because early identification of relapse has not been proven to improve outcomes. The utility of surveillance imaging in lymphomas has long been debated for the same reason. An ongoing prospective study led by Memorial Sloan Kettering Cancer Center is comparing a prespecified CT surveillance schedule to every-3-month MRD analysis (using Ig-HTS) in DLBCL patients in first remission (NCT02633111).[57] The results of this important study should help to better define the utility of ctDNA MRD analysis as a surveillance strategy.

Pre-Emptive Therapy to Prevent Clinical Relapse

In addition to simply detecting impending relapse (see above), MRD analysis can be used as a trigger to initiate preemptive therapy designed to prevent overt clinical relapse. It is hoped that such early intervention will translate into improved outcomes for patients. In 2 consecutive prospective trials by the Nordic Lymphoma Group (MCL2 and MCL3), MCL patients underwent intensive first-line therapy that included auto-HCT.[53,58] Post-transplant, patients with a molecular marker of disease were monitored for MRD using ASO-PCR. Patients who became MRD-positive were eligible for preemptive treatment with rituximab (375 mg/m^2 weekly × 4 weeks). Combining the results of the MCL2 and MCL3 patients, Kolstad and colleagues reported that, of 92 total preemptive rituximab courses (25 of 58 patients received preemptive rituximab courses more than once),

80 (87%) led to reattainment of MRD negativity. In addition, among rituximab-treated patients, at a median follow-up of 55 months after rituximab, 48% remained in clinical remission. Considering that development of MRD positivity typically leads to overt clinical relapse in a matter of a few months in MCL patients, the results of this study suggest that preemptive rituximab is an effective strategy to treat molecular relapse, and that this strategy delays overt clinical relapse.[59] More recently, a survival benefit has been demonstrated using rituximab maintenance therapy after auto-HCT in MCL patients.[60] In many parts of the world, MCL patients receive rituximab maintenance (1 dose every 8 weeks for 3 years) following auto-HCT regardless of MRD status, because this has been associated with a survival benefit.[60] It is unclear whether monitoring MRD during rituximab maintenance has value, although one could envision a preemptive strategy in which a targeted agent such as a Bruton tyrosine kinase (BTK) inhibitor could be added to patients who become MRD-positive while on rituximab maintenance. In addition, once patients complete maintenance rituximab, there may be value in monitoring MRD with the intention of initiating preemptive therapy with rituximab or possibly with another agent, such as a BTK inhibitor.

As discussed above, following allogeneic HCT, ctDNA was able to predict clinical relapse in 84% of patients, with an average lead time of 3.7 months.[56] As a result, this would provide an opportunity for preemptive therapy in this setting, which could consist of donor lymphocyte infusion, checkpoint inhibition, or chimeric antigen receptor (CAR) T-cell therapy.

Risk-Adapted First-Line Therapy

ctDNA assessment is ideally suited for developing a risk-adapted approach to first-line therapy, in which some patients receive intensified treatment and others less-intensive treatment, based on MRD analysis at a particular time.

In one possible design, only patients who showed early reduction or negative ctDNA at a particular time in response to the therapeutic regimen would continue to receive that treatment. In contrast, patients who remained MRD-positive would have their treatment intensified or changed in hopes of avoiding overt clinical relapse/progression and delay in commencing the next line of therapy. In another possible design, therapy would be de-escalated in patients who achieved an MRD-negative remission at a certain time. This would hopefully allow for good disease control while avoiding potential toxicities of more aggressive treatment. For either design, it is important to utilize an MRD assay that is both highly sensitive and highly specific.

In the United States, the EA4151 study (Rituximab With or Without Stem Cell Transplant in Treating Patients With Minimal Residual Disease-Negative Mantle Cell Lymphoma in First Complete Remission; NCT03267433) led by the ECOG-ACRIN Cancer Research Group is investigating such a risk-adapted approach. In this study, MCL patients aged 70 or younger undergo induction therapy, using any induction regimen their treating physician prefers. At the end of induction, patients are assessed by ^{18}F-FDG PET/CT, bone marrow biopsy, and Ig-HTS MRD. Patients who are not in CR, or those who remain MRD-positive, proceed to standard-of-care treatment with auto-HCT followed by 3 years of rituximab maintenance. Patients who are in an MRD-negative CR are randomly assigned to either (1) auto-HCT followed by 3 years of rituximab maintenance, or (2) 3 years of rituximab maintenance with deferral of auto-HCT. The hypothesis is that patients who are MRD-negative are already in a deeper remission state and will benefit less from high-dose therapy with auto-HCT in first remission. The study is powered to detect a survival difference at 6 years post-induction, with a secondary endpoint of PFS at 4 years post-induction.[61] This study is currently open and accruing at numerous academic and community sites across the United States. Patients can be enrolled after starting induction therapy. In addition, induction therapy as well as maintenance rituximab can be given locally by the patient's community oncologist, even if the study is not open at that site.

Following Clonal Evolution and Tailoring Therapy

As discussed earlier, the newer NGS-based techniques—in particular, hybrid capture–based NGS—allow for genotyping of lymphoma and account for tumor heterogeneity, thereby serving as liquid biopsies. Multiple mutations with allele frequencies of greater than 20% can be identified and tracked reliably.[35,37] In diseases in which driver mutations have not yet been identified, such as marginal zone lymphoma, tracking clonal evolution with liquid biopsy can help better understand the underlying biology. Moreover, serial ctDNA assessments can detect the emergence of mutations that confer drug resistance to chemotherapy, as well as targeted therapy such as ibrutinib.[37,49,50] Real-time genotyping data could be extremely valuable in personalizing a treatment approach with agents targeted to the mutations detected, and also for incorporating modifications of treatment based on the changing mutational profile prior to overt clinical progression. However, whether such an approach results in improved clinical outcomes has yet to be demonstrated. Furthermore, at its present developmental stage, ctDNA cannot accurately differentiate de novo DLBCL from transformed tumors

or fully detect chromosomal translocations, which are easily identified by a tumor tissue biopsy.[35,37] It also cannot identify mutations with low allele frequency, which may be important in certain situations (eg, TP53 or MYC).

Conclusions

MRD assessment using NGS of ctDNA is a highly sensitive and specific method to assess disease burden in lymphoma patients. ctDNA MRD analysis can provide information regarding prognosis, response to treatment, and detection of relapse prior to overt clinical relapse. Monitoring ctDNA could allow for a reduction or complete elimination of surveillance imaging, while still obtaining a quantitative assessment of disease burden over time. MRD analysis of ctDNA also provides the opportunity for early (preemptive) therapy at the time of molecular relapse. In addition, MRD analysis lends itself to customized or risk-adapted therapy, such that those at higher risk receive more intensive therapy, whereas those at lower risk can avoid the toxicities of such intensification. Several ongoing trials will hopefully clarify the optimal use of ctDNA MRD analysis in the management of lymphoma patients. This should ultimately improve outcomes by allowing customized therapy, permitting early intervention before overt relapse, and reducing radiation exposure from imaging during surveillance.

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