Established in 1871

Swiss Medical Weekly

Formerly: Schweizerische Medizinische Wochenschrift

An open access, online journal • www.smw.ch

Review article: Biomedical intelligence | Published 23 January 2019 | doi:10.4414/smw.2019.14709
Cite this as: Swiss Med Wkly. 2019;149:w14709

Liquid biopsy in tissue-born lymphomas

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Summary

In the era of personalised medicine, genetic information is critical to directing therapeutic options, aiding risk stratification and disease monitoring of lymphomas. Liquid biopsy is a novel noninvasive, real-time and tumour-specific technique, reliably reflecting the comprehensive tumour genetic profile, and thus holds great promise for the genetic assessment, response monitoring and relapse detection of lymphomas. Standard methods for disease response assessment in patients with lymphoma, including positron emission tomography, are imperfect. In other haematological malignancies, particularly leukaemias, the ability to detect minimal residual disease (MRD) is increasingly influencing treatment paradigms. However, in some subtypes of lymphoma, such as diffuse large B-cell lymphoma and classic Hodgkin's lymphoma, the application of MRD assessment techniques such as flow cytometry or polymerase chain reaction-based methods has been challenged by the absence of circulating disease. The review summarises the applications of liquid biopsy in the assessment of tumour burden and response to therapy, noninvasive genomic profiling, and monitoring of clonal dynamics in patients with diffuse large B-cell lymphoma and classic Hodgkin's lymphoma.

Keywords: *cfDNA, next generation sequencing, lymphoma, liquid biopsy, genotyping, minimal residual disease*

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The liquid biopsy

The accessing of tumour-specific DNA simply in plasma, serum or other body fluids, without the necessity of an invasive tumour biopsy, led to the concept of "liquid biopsy" in lymphoma types lacking leukaemic involvement. Cell-free fragments of DNA (cfDNA) are shed into the bloodstream by tumour cells undergoing apoptosis and circulate in plasma as double-stranded DNA fragments that are predominantly short (<200 base pairs [bp]), and normally at a low concentration [1]. In healthy individuals, plasma cfDNA is believed to derive primarily from apoptosis of normal cells of haematopoietic lineage, with minimal contributions from other tissues [2–6]. The size distribution of cfDNA fragments is highly characteristic, corresponding to nucleosomes (~147 bp) [7] and chromatosomes (nucleosome + linker histone; ~167 bp) [8, 9]. In healthy subjects, plasma cfDNA concentrations range between 1 and 10 ng/ml [10, 11]. In lymphoma patients, a proportion of cfDNA derives from apoptotic tumour cells [5]. Consistently, the total amount of cfDNA in lymphoma patients is increased compared with age and sex matched healthy subjects, with a mean concentration of 30 ng/ml of plasma [12–15].

Levels of cfDNA vary across different lymphoma subtypes. The concentrations of cfDNA in patients with untreated diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma and mantle cell lymphoma are significantly higher than in healthy controls (12.1 ng/ml), at 26.9, 25.7 and 23.1 ng/ml, respectively, whereas the concentration of cfDNA is lower in follicular lymphoma (14.7 ng/ml) [14].

Beside the type of lymphoma, tumour volume also impacts on cfDNA levels. Consistently, the median concentration of cfDNA in early stage DLBCL patients is significantly lower than in advanced stage cases [12]. By using the immunoglobulin gene rearrangement or the tumour mutation profile as lymphoma fingerprints, it is possible to discriminate normal cfDNA from cfDNA derived from tumour cells, also called circulating tumour DNA (ctDNA) [12, 15–18]. Genomic analysis and quantification of ctDNA in lymphoma patients allows: (i) the identification of tumour mutations without need of a biopsy; (ii) tracking of tumour clonal evolution and identification of mutations causing resistance to treatment; and (iii) monitoring of residual disease after therapy.

Monitoring tumour immunoglobulin heavy chain (*IgH*) gene rearrangement on circulating tumour DNA

To evaluate lymphoma-specific ctDNA, several groups have analysed circulating clonal *IgH* gene rearrangements utilising PCR, high-throughput sequencing or next generation sequencing (NGS) [12, 13, 15, 19]. In these studies, the majority of patients had measurable *IgH* rearrangements in the plasma at disease presentation and the detection of plasma ctDNA *IgH* rearrangements was shown to have greater sensitivity than *IgH* rearrangements in circulating cells (fig. 1) [15]. In particular, three independent studies detected clonotypic immunoglobulin rearrangements in pretreatment ctDNA in 82 to 92% of cases [12, 13, 15]. Levels of ctDNA correlated with other measures of tumour burden, including lactate dehydrogenase, metabolic tumour volume and International Prognostic Index. Furthermore, they suggested a prognostic role for monitor-

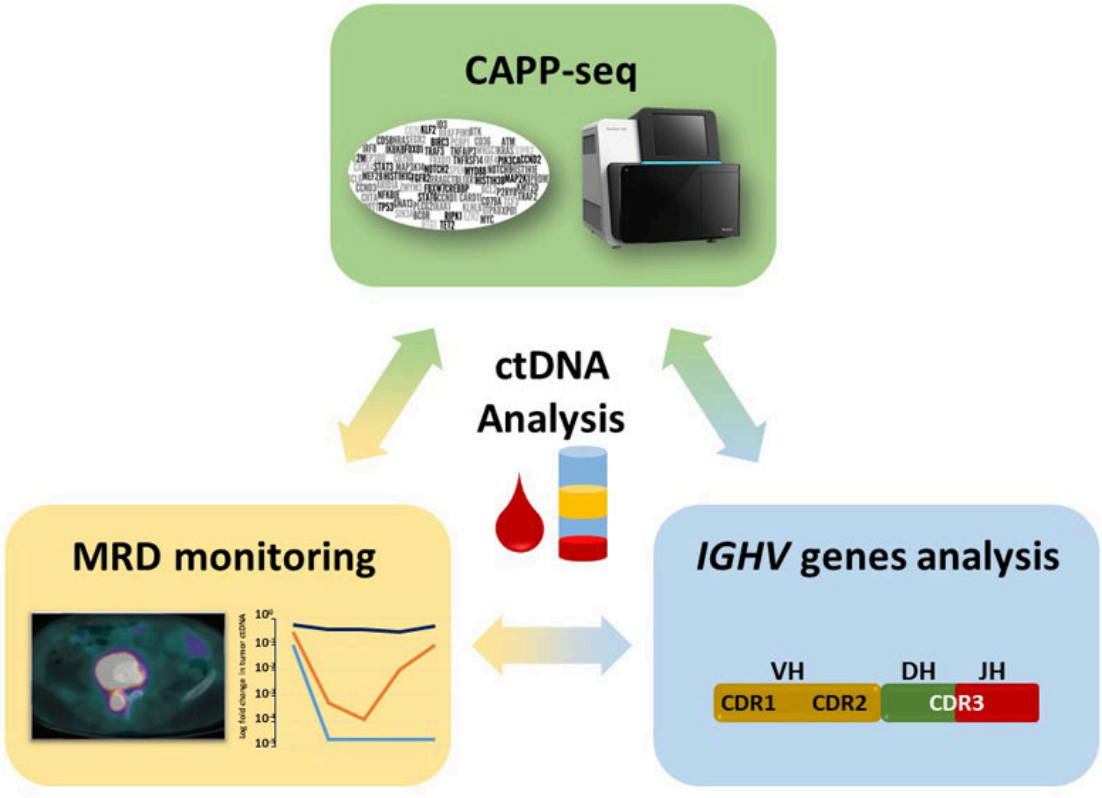
ing ctDNA during and after therapy. In patients achieving an initial complete remission, ctDNA was detectable 3 to 3.5 months before clinical relapse, outperforming positron emission tomography / computed tomography (PET/CT) [12, 15]. In addition, patients with undetectable ctDNA on the first day of cycle 3 of therapy had a superior progression-free survival, but not overall survival, compared with patients with positive ctDNA [12]. Despite its value as a prognostic tool, tracking *IgH* sequences has several shortcomings, including the need for lymphoma clonotype assignment through the analysis of the tissue biopsy, limited sensitivity in low tumour burden settings and reduced applicability because of somatic hypermutation, leading to difficulty identifying clonotypic sequences.

Tumour genotyping on circulating tumour DNA

Tumour genotyping of mature B-cell tumours lacking a leukaemic phase has so far relied on analysis of the diagnostic tissue biopsy. However, multiregional sequencing showed that the diagnostic tissue biopsy might be subject to a selection bias resulting from spatial heterogeneity and, therefore, might not be representative of the entire tumour genetics. Indeed, different areas of the same tumour may show different genetic profiles (intratumoural heterogeneity). A biopsy from one part of a tumour may miss mutations occurring in subclones residing in anatomically distant sites, including clinically relevant genetic biomarkers for treatment tailoring or anticipation of resistance [20]. Furthermore, serial sampling of tumour material through repeat biopsies is usually not feasible in mature B-cell malignancies lacking a leukaemic phase, which hampers efforts to understand patterns of genomic evolution during disease progression and limits the monitoring of minimal residual disease (MRD) and the development of treatment emergent resistant mutations. On these bases, approaches that are complementary to the analysis of the diagnostic tissue biopsy are required in order to deal with the clinical need of comprehensive and easily accessible tumour genotyping. Recent advances in the sensitivity and accuracy of DNA analysis have allowed genotyping of ctDNA for the identification of somatic cancer gene mutations [21]. The ability to detect and quantify tumour mutations has proven effective in tracking tumour dynamics in real time, as well as serving as a liquid biopsy that can be used for a variety of clinical and investigational applications not previously possible. As ctDNA is representative of the entire tumour heterogeneity, it bypasses the bias imposed by tissue biopsies in the reconstruction of the entire cancer clonal architecture and allows identification of resistant clones that are dormant in inaccessible tumour sites. Accessing the blood stream has also a clear sampling advantage in the serial monitoring of treatment emergent resistant mutations in real time [21].

A sensitive method used to detect disease-specific mutations in ctDNA is the CAncer Personalised Profiling by deep Sequencing (CAPP-seq) (fig.1) [22, 23]. CAPPseq utilises a disease-specific "selector", which is a set of exonic and intronic targets chosen to cover regions of known recurrent mutations in a particular cancer type. Those targets are then amplified and sequenced in a patient's plasma sample, allowing quantification of ctDNA based on the

Figure 1: Molecular applications of plasma circulating tumour DNA (ctDNA).



detection of tumour-specific mutations, and simultaneous determination of an individual's specific tumour mutation profile. This method can simultaneously test for all important classes of mutation, including single nucleotide variants (SNVs), insertion/deletions (indels), copy number alterations and rearrangements. CAPP-seq has been used in highly promising studies in both solid and haematological malignancies [22–24], based on its high sensitivity and ability to reliably identify patient-specific target sequences.

Independent studies assessed targeted gene mutations in ctDNA from untreated DLBCL patients by use of CAPP-Seq. Our recent study has recently contributed to the development of this highly sensitive ultra-deep NGS approach to track DLBCL genetic profiles using plasma ctDNA [18]. Genotyping of 50 pretreated DLBCL plasma samples, which interrogated 59 genes, correctly identified almost all the mutations that were represented in the lymphoma tissue biopsy with a 92% detection specificity of tumour biopsy-confirmed mutations in ctDNA [18]. In a second study of 37 pretreated DLBCL plasma samples, which interrogated 268 genes, the detection specificity of tumour biopsy-confirmed mutations in ctDNA was 99.3%, variants were identified in 97% of cases, and confirmed a median of 95% of SNVs per ctDNA sample in paired tumour biopsies, indicating that plasma ctDNA is an effective surrogate for direct tumour genotyping [25].

A third study of 45 pretreated DLBCL plasma samples detected ctDNA in 100% of patients with 99.8% specificity when tumour genotypes were known. In addition, 91% of tumour-confirmed SNVs in driver genes could be genotyped directly from pretreatment ctDNA, and this detection rate was directly correlated with ctDNA concentrations [26]. Finally, a study of 12 diagnostic DLBCL plasma samples identified a median concordance mutational rate of 85% between tumour DNA and ctDNA by using a specific Lymphopanel of 34 genes [27]. The CAPP-Seq approach was also applied and validated in other mature B-cell malignancies lacking a leukaemic phase. In our recent study, we established ctDNA as an alternative and accessible source of tumour DNA for classic Hodgkin's lymphoma (cHL) genotyping [28]. By identifying *STAT6* as the most frequently mutated gene in ~ 40% of cases, we refined the current knowledge of cHL genetics. Longitudinal ctDNA profiling identified treatment-dependent patterns of clonal evolution in patients relapsing after chemotherapy and patients maintained in partial remission under immunotherapy [28].

A recent proof-of-concept study reported, for the first time, that the detection and measurement by digital PCR of recurrent somatic mutations in the plasma ctDNA of patients with DLBCL is possible, easy, and reproducible [29]. Testing of ctDNA with digital PCR is emerging as an appropriate and helpful molecular tool for the management of DLBCL, with NGS methods. A recent retrospective study including 94 all-stage cHL patients undergoing first-line therapy demonstrated, using dPCR, the presence of a highly recurrent exportin 1 (*XPO1*) E571K mutation in 24.2% of the patients [30]. A second study analysed ctDNA from 14 primary central nervous system lymphoma patients at diagnosis by using droplet digital PCR (ddPCR) and targeted deep sequencing. The group identified the *MYD88* L265P mutation in 100% of cases by using ddPCR and 0% of cases by using targeted deep sequencing, implying dependence on the detection method [31]. Finally, a second recent study of nine diagnosed intravascular large B-cell lymphoma patients investigated a sequencing panel of eight genes by targeted sequencing with a mutation detection sensitivity in ctDNA of 100% compared with paired tumour tissue. The mutation detection sensitivity was also confirmed when they investigated mutation of *MYD88* L265P by means of ddPCR assay [32].

Through these novel methods, disease-specific mutations can be detected in ctDNA at diagnosis and followed throughout the course of the disease to quantitatively monitor tumour dynamics during treatment and to detect emergent mutations that may represent clonal evolution and/or herald resistance to targeted therapy.

Minimal residual disease monitoring of circulating tumour DNA in diffuse large B-Cell lymphoma

Owing to a lack of leukaemic involvement, MRD monitoring has, so far, been limited in tissue-born lymphomas such as DLBCL. In this lymphoma MRD cannot be determined by conventional methods, which hampers monitoring of MRD at the end of and after induction treatment. Against this background, ctDNA holds great potential for detection of MRD below the detection threshold of PET/CT (fig.1). However, to date few studies have assessed the clinical value of liquid biopsy at the end of treatment in DLBCL. One study tested plasma DNA in seven DLBCL patients who achieved complete remission on PET/CT after four cycles of treatment, and all seven cases had undetectable ctDNA, consistent with the PET/CT results [15]. In a second study, researchers analysed ctDNA from 17 patients whose disease progressed after achieving complete remission at the end of initial treatment, and they found positive ctDNA in 15 patients before progression [12]. Taken together, these results suggest that ctDNA analysis may be utilised in a manner that is complementary to PET imaging for residual disease monitoring.

Minimal residual disease monitoring on circulating tumour DNA in classical Hodgkin's lymphoma

Limited access to tumour material from the tissue biopsy has hampered the development of molecular prognostic and predictive factors in cHL, a frequent and clinically heterogeneous mature B-cell tumour whose stratification represents an unmet medical need. The rarity (usually <5%) of tumour cells (Hodgkin and Reed-Sternberg cells) in biopsies of lymph nodes involved by cHL and their routine formalin-fixed paraffin-embedded preservation impose major technical hurdles, and the identification of genetic features for disease monitoring and response assessment is challenging in this disease. In a large study using a highly sensitive NGS method, lymphoma-specific immunoglobulin gene segments were identified in cHL at initial diagnosis or at recurrence, and the ability to detect such lymphoma-specific sequences in peripheral blood was assessed [33]. Furthermore, pivotal evidence suggests that the use of ctDNA for tumour genotyping may allow such technical issues in cHL to be bypassed [34]. In a retrospective study, cHL

patients with a detectable *XPO1* mutation in plasma ctDNA detected with digital PCR at the end of treatment had a shorter progression-free survival than *XPO1* negative cases [30]. These results support clearance of the *XPO1* mutation in plasma ctDNA during and at the end of treatment as a new prognostic marker for patients with detectable mutations [30].

In our recent report of ctDNA changes during therapy, we proposed ctDNA as a radiation-free tool to track residual disease, which may integrate PET imaging for the early identification of chemorefractory cHL patients and provide proof of concept that ctDNA may serve as novel precision-medicine biomarker in cHL [28].

Potential applications of liquid biopsies

Liquid biopsy is specific, efficient and less invasive than tissue biopsy for patients. The technology could also aid in treatment selection during routine clinical care, monitoring of medication effects such as drug resistance or tumour evolution, identification of recurrent or minimal residual disease and, ideally, detection of cancers in their most nascent stages and informing prognoses [25, 26]. The same benefits are applicable in the clinical trial setting, including screening patients for trial enrolment. The potential of liquid biopsies to detect changes in tumour genetics, before imaging reveals changes in growth, could enable therapy modifications or earlier second-line interventions [28].

The most immediate implementations of ctDNA technology in clinical trials include: (i) baseline screening to identify patients harbouring actionable mutations (e.g., the study aimed at addressing tazemetostat in EZH2 mutated follicular lymphoma [35]); (ii) early and accurate identification, by combining interim PET/CT with ctDNA measurement, of nonresponding patients who can be enrolled in intensification or biological agent-based treatment approaches [28, 36]; (iii) monitoring the development of resistance mutations against the targeted agent tested in the trial.

Conclusions

The liquid biopsy approach has a great potential for the detection and surveillance of disease relapse, and many choices for the quantitation of mutations or VDJ rearrangements in circulating tumour DNA are currently available (fig.1). Despite the fact that many of these techniques have been developed as valuable, noninvasive, appropriate and real-time monitoring tools, none has yet translated into the clinical practice.

Undoubtedly, future studies to investigate whether monitoring of ctDNA can be used to improve clinical outcomes for patients with aggressive B-cell lymphomas are warranted. New opportunities that go beyond assessment of VDJ recombination are also being explored, and ultrasensitive assays that can assess for genotypic tumour DNA in the blood are under development as well [15, 18, 23, 25]. Molecular monitoring of ctDNA holds tremendous promise and may be a transformative tool for monitoring therapy in aggressive B-cell lymphomas. It is critical that well-designed trials validate current concepts and further explore applications for interim monitoring, surveillance monitoring and response assessment [37]. In order to meet clinical standards and to distinguish true variants from sequencing errors, NGS has to be accurate and robust [38]. NGS technologies are now applied to obtain a more comprehensive view of entire genomic regions. With these technologies, NGS provides opportunities to characterise personalised cancer gene maps and develop personalised medicine. Furthermore, several solutions to increase the sensitivity of the technology have been described, such as the use of complex barcoding strategies, which enable the separation of true SNVs from errors [39–42].

Recent studies on the analysis of different sources of liquid biopsies in peripheral blood, such exosomes/extracellular vesicles and circulating tumour cells, are becoming a powerful tool in furthering our understanding of cancer biology and improving the management and survival of cancer patients [43, 44], although the biology and clinical implications of circulating tumour cells and exosomes in lymphomas are yet to be understood.

Disclosure statement

No financial support and no other potential conflict of interest relevant to this article was reported.

References

1 Fleischhacker M, Schmidt B. Circulating nucleic acids (CNAs) and cancer--a survey. Biochim Biophys Acta. 2007;1775(1):181–232. PubMed.
2 Thierry AR, El Messaoudi S, Gahan PB, Anker P, Stroun M. Origins, structures, and functions of circulating DNA in oncology. Cancer Metastasis Rev. 2016;35(3):347–76. doi: http://dx.doi.org/10.1007/s10555-016-9629-x. PubMed.
3 Thakur BK, Zhang H, Becker A, Matei I, Huang Y, Costa-Silva B, et al. Double-stranded DNA in exosomes: a novel biomarker in cancer detection. Cell Res. 2014;24(6):766–9. doi: http://dx.doi.org/10.1038/cr.2014.44. PubMed.
4 Kahlert C, Melo SA, Protopopov A, Tang J, Seth S, Koch M, et al. Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. J Biol Chem. 2014;289(7):3869–75. doi: http://dx.doi.org/10.1074/jbc.C113.532267. PubMed.
5 Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res. 2001;61(4):1659–65. PubMed.
6 Stroun M, Lyautey J, Lederrey C, Olson-Sand A, Anker P. About the possible origin and mechanism of circulating DNA: apoptosis and active DNA release. Clin Chim Acta. 2001;313(1-2):139–42. doi: http://dx.doi.org/10.1016/S0009-8981(01)00665-9. PubMed.
7 Giacona MB, Ruben GC, Iczkowski KA, Roos TB, Porter DM, Sorenson GD. Cell-free DNA in human blood plasma: length measurements in patients with pancreatic cancer and healthy controls. Pancreas. 1998;17(1):89–97. doi: http://dx.doi.org/10.1097/00006676-199807000-00012. PubMed.
8 Lo YM, Chan KC, Sun H, Chen EZ, Jiang P, Lun FM, et al. Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. Sci Transl Med. 2010;2(61): 61–91. doi: http://dx.doi.org/10.1126/scitranslmed.3001720. PubMed.
9 Thierry AR, Mouliere F, Gongora C, Ollier J, Robert B, Ychou M, et al. Origin and quantification of circulating DNA in mice with human colorectal cancer xenografts. Nucleic Acids Res. 2010;38(18):6159–75. doi: http://dx.doi.org/10.1093/nar/gkq421. PubMed.
10 Mouliere F, El Messaoudi S, Pang D, Dritschilo A, Thierry AR. Multimarker analysis of circulating cell-free DNA toward personalized medicine for colorectal cancer. Mol Oncol. 2014;8(5):927–41. doi: http://dx.doi.org/10.1016/j.molonc.2014.02.005. PubMed.
11 Mouliere F, Robert B, Arnau Peyrotte E, Del Rio M, Ychou M, Molina F, et al. High fragmentation characterizes tumour-derived circulating DNA. PLoS One. 2011;6(9):. doi: http://dx.doi.org/10.1371/journal.pone.0023418. PubMed.
12 Roschewski M, Dunleavy K, Pittaluga S, Moorhead M, Pepin F, Kong K, et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. Lancet Oncol. 2015;16(5):541–9. doi: http://dx.doi.org/10.1016/S1470-2045(15)70106-3. PubMed.

13 Armand P, Oki Y, Neuberg DS, Faham M, Cummings C, Klinger M, et al. Detection of circulating tumour DNA in patients with aggressive B-cell non-Hodgkin lymphoma. Br J Haematol. 2013;163(1):123–6. doi: http://dx.doi.org/10.1111/bjh.12439. PubMed.

14 Hohaus S, Giachelia M, Massini G, Mansueto G, Vannata B, Bozzoli V, et al. Cell-free circulating DNA in Hodgkin's and non-Hodgkin's lymphomas. Ann Oncol. 2009;20(8):1408–13. doi: http://dx.doi.org/10.1093/annonc/mdp006. PubMed.

15 Kurtz DM, Green MR, Bratman SV, Scherer F, Liu CL, Kunder CA, et al. Noninvasive monitoring of diffuse large B-cell lymphoma by immunoglobulin high-throughput sequencing. Blood. 2015;125(24):3679–87. doi: http://dx.doi.org/10.1182/blood-2015-03-635169. PubMed.

16 Forshew T, Murtaza M, Parkinson C, Gale D, Tsui DW, Kaper F, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Transl Med. 2012;4(136):. doi: http://dx.doi.org/10.1126/scitranslmed.3003726. PubMed.

17 Ladetto M, Brüggemann M, Monitillo L, Ferrero S, Pepin F, Drandi D, et al. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. Leukemia. 2014;28(6):1299–307. doi: http://dx.doi.org/10.1038/leu.2013.375. PubMed.

18 Rossi D, Diop F, Spaccarotella E, Monti S, Zanni M, Rasi S, et al. Diffuse large B-cell lymphoma genotyping on the liquid biopsy. Blood. 2017;129(14):1947–57. doi: http://dx.doi.org/10.1182/blood-2016-05-719641. PubMed.

19 Frickhofen N, Müller E, Sandherr M, Binder T, Bangerter M, Wiest C, et al. Rearranged Ig heavy chain DNA is detectable in cell-free blood samples of patients with B-cell neoplasia. Blood. 1997;90(12):4953–60. PubMed.

20 Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366(10):883–92. doi: http://dx.doi.org/10.1056/NEJMoa1113205. PubMed.

21 Diaz LA, Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol. 2014;32(6):579–86. doi: http://dx.doi.org/10.1200/JCO.2012.45.2011. PubMed.

22 Kurtz DM, Scherer F, Newman AM, Craig AFM, Khodadoust MS, Lovejoy AF, et al. Prediction of therapeutic outcomes in DLBCL from circulating tumor DNA dynamics. In: 2016 ASCO Annual Meeting, Abstract, 175, 3679–3687.

23 Newman AM, Bratman SV, To J, Wynne JF, Eclov NCW, Modlin LA, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med. 2014;20(5):548–54. doi: http://dx.doi.org/10.1038/nm.3519. PubMed.

24 Scherer F, Kurtz DM, Newman AM, Shahrokh Esfahani M, Craig A, Stehr H, et al. Development and validation of biopsy-free genotyping for molecular subtyping of diffuse large B-cell lymphoma. Blood. 2016;128:1089.

25 Scherer F, Kurtz DM, Newman AM, Stehr H, Liu CL, Zhou L, et al. Non invasive genotyping and assessment of treatment response in diffuse large B cell lymphoma. Blood. 2015;126(23):114.

26 Scherer F, Kurtz DM, Newman AM, Stehr H, Craig AF, Esfahani MS, et al. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. Sci Transl Med. 2016;8(364):. doi: http://dx.doi.org/10.1126/scitranslmed.aai8545. PubMed.

27 Bohers E, Viailly PJ, Dubois S, Bertrand P, Maingonnat C, Mareschal S, et al. Somatic mutations of cell-free circulating DNA detected by next-generation sequencing reflect the genetic changes in both germinal center B-cell-like and activated B-cell-like diffuse large B-cell lymphomas at the time of diagnosis. Haematologica. 2015;100(7):e280–4. doi: http://dx.doi.org/10.3324/haematol.2015.123612. PubMed.

28 Spina V, Bruscaggin A, Cuccaro A, Martini M, Di Trani M, Forestieri G, et al. Circulating tumor DNA reveals genetics, clonal evolution, and residual disease in classical Hodgkin lymphoma. Blood. 2018;131(22):2413–25. doi: http://dx.doi.org/10.1182/blood-2017-11-812073. PubMed.

29 Camus V, Sarafan-Vasseur N, Bohers E, Dubois S, Mareschal S, Bertrand P, et al. Digital PCR for quantification of recurrent and potentially actionable somatic mutations in circulating free DNA from patients with diffuse large B-cell lymphoma. Leuk Lymphoma. 2016;57(9):2171–9. doi: http://dx.doi.org/10.3109/10428194.2016.1139703. PubMed.

30 Camus V, Stamatoullas A, Mareschal S, Viailly PJ, Sarafan-Vasseur N, Bohers E, et al. Detection and prognostic value of recurrent exportin 1 mutations in tumor and cell-free circulating DNA of patients with classical Hodgkin lymphoma. Haematologica. 2016;101(9):1094–101. doi: http://dx.doi.org/10.3324/haematol.2016.145102. PubMed.

31 Hattori K, Sakata-Yanagimoto M, Suehara Y, Yokoyama Y, Kato T, Kurita N, et al. Clinical significance of disease-specific *MYD88* mutations in circulating DNA in primary central nervous system lymphoma. Cancer Sci. 2018;109(1):225–30. doi: http://dx.doi.org/10.1111/cas.13450. PubMed.

32 Suehara Y, Sakata-Yanagimoto M, Hattori K, Nanmoku T, Itoh T, Kaji D, et al. Liquid biopsy for the identification of intravascular large B-cell lymphoma. Haematologica. 2018;103(6):e241–4. doi: http://dx.doi.org/10.3324/haematol.2017.178830. PubMed.

33 Herrera AF, Kim HT, Kong KA, Faham M, Sun H, Sohani AR, et al. Next-generation sequencing-based detection of circulating tumour DNA After allogeneic stem cell transplantation for lymphoma. Br J Haematol. 2016;175(5):841–50. doi: http://dx.doi.org/10.1111/bjh.14311. PubMed.

34 Schmitt MW, Kennedy SR, Salk JJ, Fox EJ, Hiatt JB, Loeb LA. Detection of ultra-rare mutations by next-generation sequencing. Proc Natl Acad Sci USA. 2012;109(36):14508–13. doi: http://dx.doi.org/10.1073/pnas.1208715109. PubMed.

35 Morschhauser F, Salles G, McKay P, Tilly H, Schmitt A, Gerecitano J, et al. Interim report from a phase 2 multicenter study of tazemetostat, an EZH2 inhibitor, in patients with relapsed or refractory B-cell non-Hodgkin Lymphomas. Hematol Oncol. 2017;35(S2):24–5. [Abstract 4]. doi: http://dx.doi.org/10.1002/hon.2437_3.

36 Kurtz DM, Scherer F, Jin MC, Soo J, Craig AFM, Esfahani MS, et al. Circulating Tumor DNA Measurements As Early Outcome Predictors in Diffuse Large B-Cell Lymphoma. J Clin Oncol. 2018;36(28):2845–53. doi: http://dx.doi.org/10.1200/JCO.2018.78.5246. PubMed.

37 Roschewski M, Staudt LM, Wilson WH. Dynamic monitoring of circulating tumor DNA in non-Hodgkin lymphoma. Blood. 2016;127(25):3127–32. doi: http://dx.doi.org/10.1182/blood-2016-03-635219. PubMed.

38 Mattocks CJ, Morris MA, Matthijs G, Swinnen E, Corveleyn A, Dequeker E, et al.; EuroGentest Validation Group. A standardized framework for the validation and verification of clinical molecular genetic tests. Eur J Hum Genet. 2010;18(12):1276–88. doi: http://dx.doi.org/10.1038/ejhg.2010.101. PubMed.

39 Kennedy SR, Schmitt MW, Fox EJ, Kohrn BF, Salk JJ, Ahn EH, et al. Detecting ultralow-frequency mutations by Duplex Sequencing. Nat Protoc. 2014;9(11):2586–606. doi: . Correction in: Nat Protoc. 2014;9:2903. doi:https://doi.org/10.1038/nprot1214-2903d. http://dx.doi.org/10.1038/nprot.2014.170. PubMed.

40 Hiatt JB, Pritchard CC, Salipante SJ, O'Roak BJ, Shendure J. Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation. Genome Res. 2013;23(5):843–54. doi: http://dx.doi.org/10.1101/gr.147686.112. PubMed.

41 Kinde I, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B. Detection and quantification of rare mutations with massively parallel sequencing. Proc Natl Acad Sci USA. 2011;108(23):9530–5. doi: http://dx.doi.org/10.1073/pnas.1105422108. PubMed.

42 Peng Q, Vijaya Satya R, Lewis M, Randad P, Wang Y. Reducing amplification artifacts in high multiplex amplicon sequencing by using molecular barcodes. BMC Genomics. 2015;16(1):589. doi: http://dx.doi.org/10.1186/s12864-015-1806-8. PubMed.

43 Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. Expert Rev Proteomics. 2009;6(3):267–83. doi: http://dx.doi.org/10.1586/epr.09.17. PubMed.

44 Heitzer E, Auer M, Ulz P, Geigl JB, Speicher MR. Circulating tumor cells and DNA as liquid biopsies. Genome Med. 2013;5(8):73. doi: http://dx.doi.org/10.1186/gm477. PubMed.