

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

**WEB OF SCIENCE™**Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com

Introductory Chapter: Liquid Biopsy – A Promising Technology of the Future

Ilze Strumfa and Janis Gardovskis

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.86918>

1. Introduction

Liquid biopsy represents a spectrum of technologies for the molecular analysis of blood and other biological liquids (e.g. urine, saliva or others) in order to detect cell-free/tumour nucleic acids, exosomes, microRNAs, tumour-educated platelets and circulating or free-floating tumour or foetal cells, depending on the clinical context. In contrast to classic tumour markers, e.g. prostate-specific antigen (PSA) or carcinoembryonic antigen (CEA), liquid biopsy is distinguished by high specificity as it provides genomic, proteomic and cellular characteristics of the disease. The anticipated outstanding reliability of these tests has been reflected in the term itself: “liquid biopsy” is expected to be at least as informative as tissue biopsy, used as the gold standard in certain diagnostic fields, especially in oncology.

The advantages of liquid biopsy include non-invasive approach which is patient-friendly, associated with remarkably low possibility of complications and technically feasible even in patients who are in serious general status or affected by tumour or metastases that are not easily accessible by conventional tissue biopsy. The clinically simple application allows the repeated use of liquid biopsy resulting in real-time follow-up for the disease course. The testing is fast and exact as definite molecular markers are sought for. Generally, liquid biopsy is a much awaited tool to overcome the limitations set by tumour heterogeneity upon conventional tissue biopsy representing only a small part of the whole tumour [1]. However, some technological modifications of liquid biopsy in certain patients can be subjected to the same restrictions regarding heterogeneity. A characteristic example would be the assessment of circulating tumour cells (CTCs) in patients with low burden of malignant cells in the blood. For instance, the diagnostic threshold of

two CTCs per 7.5 mL of blood ensured high sensitivity and specificity for gastric cancer diagnosis, reaching 85.3 and 90.3%, respectively [2]. Thus, the diagnostic value of liquid biopsy in such setting has proven to be high, but the few malignant cells per sample cannot represent the full scope of cancer heterogeneity, by the number being inferior to tissue biopsy. Similarly, to characterize the tumour exactly, the circulating tumour DNA must represent at least 10% of the whole blood burden of cell-free DNA – a threshold that is not always reached [1].

Liquid biopsies are increasingly applied in the evaluation of oncological patients (**Figure 1**) due to the previously listed benefits over conventional tissue biopsies. However, this approach is not limited to the diagnostics of malignant tumours [3, 4].

In addition to the increasing use in oncology, liquid biopsy can be used in obstetrics and gynaecology to evaluate the molecular characteristics of foetus [3, 4]; in transplantology for the early detection of graft rejection; in haemodialysis and critical care medicine to identify tissue damage; and in rheumatology, e.g. to assess systemic lupus erythematosus [4].

Currently, liquid biopsy is not a routine laboratory test in clinical practice [5], and even its clinical efficacy has been seriously questioned by the American Society of Clinical Oncology and the College of American Pathologists [6], but certain tests are approved for use or reaching the clinical life (e.g. *EGFR* gene mutation testing, approved by the Food and Drug Administration (FDA) on 2016; tumour mutation burden in blood, confirmed in 2018 as an effective tool to predict the efficacy of immunological checkpoint inhibitors), and the related field is rapidly expanding [7, 8]. The development is seen as the progress in technologies, software and quality control systems as well as diagnosis-based research to substantiate many promising clinical applications (**Figure 2**).

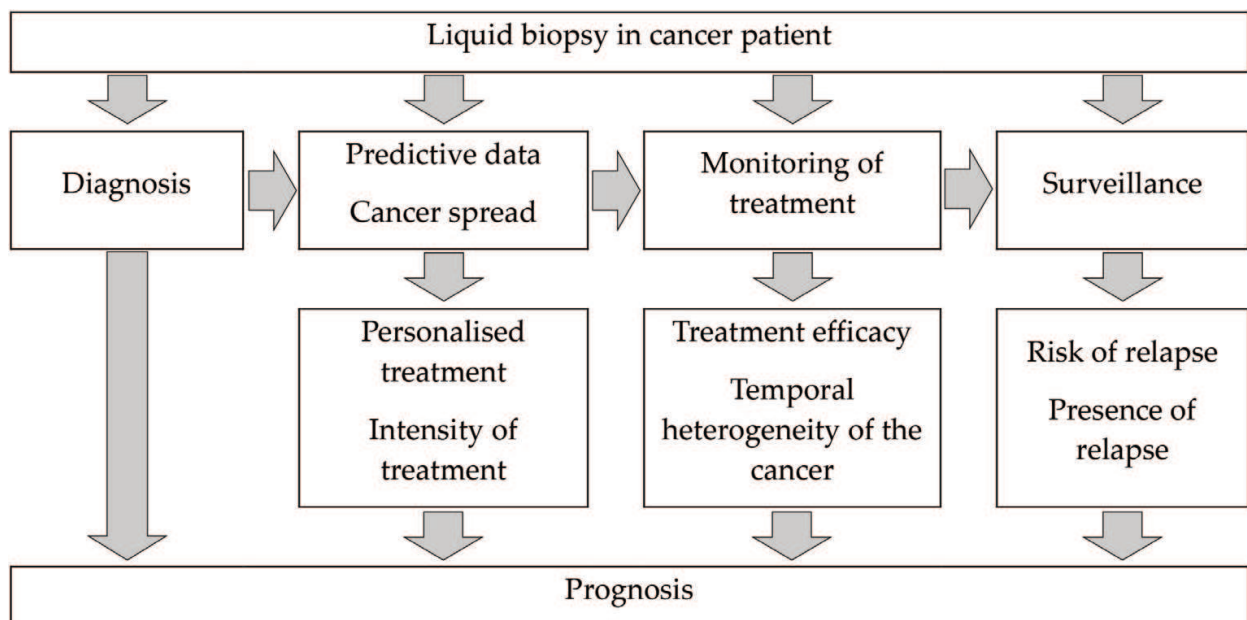


Figure 1. Role of liquid biopsy in the stepwise evaluation of cancer patient.

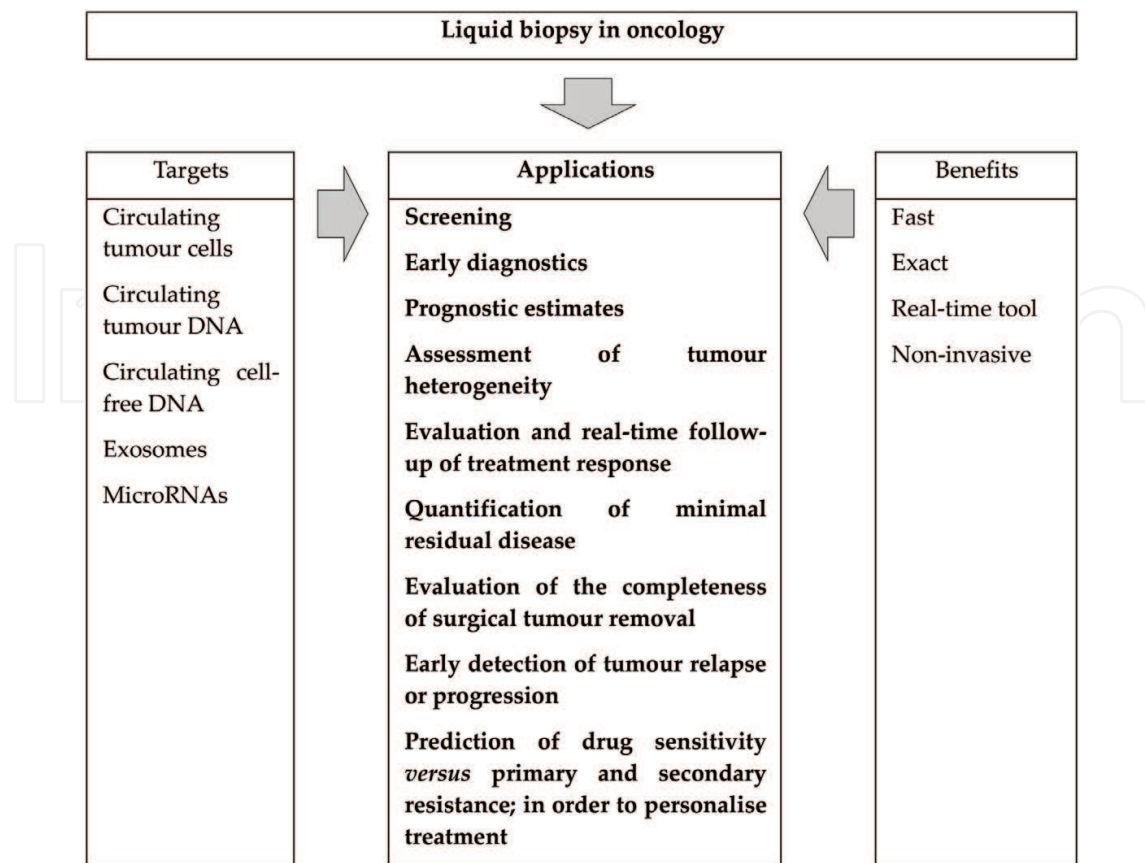


Figure 2. The potential applications of liquid biopsy in oncology.

2. Circulating tumour cells and related issues

Invasion is a typical feature of malignant tumours. An epithelial malignancy, namely, carcinoma, starts its invasive growth from intraepithelial carcinoma in situ, destroys the basement membrane, infiltrates connective tissues and reaches smaller or larger blood and lymphatic vessels, entering the circulation. Thus, at some point of cancer pathogenesis, malignant cells appear in the bloodstream. This is a crucial step of carcinogenesis, leading to the metastatic spread—the hallmark of malignant process. Circulating tumour cells (CTCs) have been described by Thomas Ashworth as long ago as in 1869 [9]. Similarly, circulating foetal cells have been identified in peripheral maternal blood [3]. However, major technological advances were necessary to develop reliable tests to identify the circulating non-haematological cells.

CTCs are generally recognised as non-leukocytic nucleated cells in the bloodstream. They can be recognised by their physical properties including large size, mechanical plasticity and dielectric mobility. Physical filters, density gradient, dielectric, microfluidic or photoacoustic methods are used for physical separation. Immunophenotype by expression of different antigens also can be applied to identify CTCs; the relevant methods include cytometric high-throughput imaging and immunomagnetic and adhesion-based separation as well as negative depletion of leukocytes and CTC identification by tumour markers [5].

Considering the pathogenesis of malignant tumours, the burden of CTCs should appear and increase in parallel with advancing cancer course. Indeed, higher numbers of circulating tumour cells were observed in patients having tumours with higher pT or pN or higher pTNM stage [10]. However, CTCs were found in more than 80% of patients presenting with pT1 and/or pN0 gastric cancer [2]—an interesting finding that has a practical value regarding the possibilities of early diagnostics but also an impact on the theoretical considerations of carcinogenesis.

Thus, CTC detection has manifold roles in oncology. A high number of CTCs is an adverse prognostic factor, shown in breast, prostatic, colorectal, gastric, pancreatic and neuroendocrine carcinomas and sarcomas as well as non-small cell lung carcinoma [5]. However, a diagnostic role has also been confirmed, e.g. in the case of breast, prostate and colorectal carcinoma [5]. Early diagnostics by CTCs has been verified in non-small cell lung carcinoma [5] and gastric cancer [2]. CTCs can provide the information on key genetic features of cancer cells and on the epigenetic changes. Hence, prediction of the treatment response by CTC has been demonstrated in breast, prostate and colorectal cancer as well as in melanoma and non-small cell lung carcinoma. Further, changes of CTC count during treatment dynamically reflects the response to treatment paralleling the residual tumour burden [5].

3. Circulating cell-free and tumour DNA

Circulating cell-free DNA (cfDNA) is present in the blood even in healthy individuals although higher levels are observed in patients diagnosed with autoimmune diseases and especially malignant tumours. Nevertheless, cfDNA seems to represent an essential biological regulatory mechanism. cfDNA is either released passively from dying—apoptotic or necrotic—cells or secreted actively from viable cells. cfDNA can be destroyed by DNase, but at least part of cfDNA pool follows another way of further biological turnover entering healthy cells. The subsequent genomic integration hypothetically can have a myriad of significant outcomes in health and disease ranging from senescence to autoimmune diseases or transfer of the cfDNA to germ cells.

In cancer patients, a fraction of circulating cell-free DNA burden is attributable to the tumour and consequently is designated as circulating tumour DNA (ctDNA). These DNA fragments are released from neoplastic cells and therefore can reflect the tumour-specific events in DNA, including somatic mutations, methylation patterns and degree of microsatellite instability. Thus, the presence of a malignant tumour can manifest by multiple quantitative and qualitative changes in circulating DNA. First, the concentration and features, e.g. fragment length of cfDNA in cancer patients, differ from healthy individuals. Second, the ctDNA reflects the specific dynamic genetic landscape of the cancer.

Evaluation of circulating DNA in certain situations can be diagnostically useful, e.g. to disclose an occult tumour. However, pitfalls exist, e.g. mutations can be present in cfDNA of healthy volunteers who do not develop cancer at least during the follow-up period. Thus, overdiagnosis of cancer by liquid biopsy must be avoided, especially when screening asymptomatic individuals. In contrast, qualitative or quantitative dynamic changes in the ctDNA of a known oncological patient bring reliable, biologically justified information. In patients with

already confirmed cancer, ctDNA can identify either minimal residual disease after surgery with curative intent or tumour relapse. Similarly, the response to treatment can be monitored. Molecular alterations can be assessed to select personalised treatment.

In addition to the diagnostic, prognostic and predictive role in oncology, cfDNA analysis might be useful in other medical situations. Thus, circulating cell-free DNA can be derived from transplanted organs or from the foetus during pregnancy, serving as an early manifestation of graft rejection or reflecting genetic features on the foetus, respectively. In addition, cfDNA levels can be valuable also as a nonspecific biomarker of tissue damage in critical care medicine and related clinical situations, e.g. sepsis, haemodialysis and others [4].

4. Circulating microRNAs

MicroRNAs (miRNAs) are small, evolutionary conserved, single-stranded, non-coding RNA molecules (approximately 22 nucleotides in length) that bind target mRNA to regulate gene expression [11, 12] at the posttranscriptional level [13]. These molecules act as large-scale molecular switches. MicroRNAs are involved in different physiological and pathological events, including apoptosis, cell proliferation and differentiation; therefore, it is not surprising to see miRNAs participating in carcinogenesis as either tumour suppressors [14, 15] or oncogenes [16]. The cardinal tumour features include cell proliferation, invasion and metastasis as well as activated angiogenesis. miRNAs regulate all the steps. In addition, up- or down regulation of certain miRNAs is associated with the biological potential of cancer, e.g. proliferation, invasivity and epithelial-mesenchymal transition or grade. miRNAs can be assessed either in tissues or in biological liquids, in the last case becoming a target for liquid biopsy.

Again, the diagnostic and regulatory roles of miRNAs are not limited to oncology. Women who develop pre-eclampsia and spontaneous preterm birth are characterised by specific exosomal miRNA profile at early gestation. Considering the interplay between exosomal secretion, oxygen tension and endothelial proliferation, aberrant exosomal signalling by placental cells is suggested to have a pathogenetic role in pregnancy complications [17].

5. Exosomes

Exosomes represent a class of extracellular vesicles mediating intercellular communication. They consist of lipid bilayer, transmembrane and non-membrane proteins and single- and/or double-stranded DNA and RNAs, including microRNAs. A fraction of exosomal proteins are ubiquitous, while others are characteristic for specific cells or tissues.

Exosomes are collected for liquid biopsy analysis by different technological approaches, including methods targeting physical (size, density, sedimentation) or antigenic properties. After the tumour-derived exosomes have been isolated and identified, exosomal protein expression and genetic profile by exosomal RNAs or DNAs or exosomal miRNA signature can be tested. In addition to cancer-produced exosomes, those originating from immune cells also can be detected and evaluated [5].

6. Tumour-educated platelets

Platelets are known to participate not only in blood clotting but also in inflammatory and immune processes, e.g. communicating with lymphocytes or regulating cellular transmigration through blood vessel's wall. In malignant tumours, platelets are involved in neoangiogenesis, induction of epithelial-mesenchymal transformation and metastatic spread of tumour cells, protecting them from the immune system. Contacting with tumour cells, platelets can uptake clinically relevant, tumour-specific biomarkers, as demonstrated in the models of glioblastoma, non-small cell lung cancer or prostate cancer. Such platelets are described as tumour-educated platelets, potentially representing diagnostic or therapeutic targets, as well as a clue to further theoretical investigations of carcinogenesis [18].

7. Conclusions

In conclusion, liquid biopsy represents molecular analysis of biological liquids in order to detect cell-free/tumour nucleic acids, exosomes and/or microRNAs, tumour-educated platelets and circulating or free-floating tumour or foetal cells. These technologies have several advantages including high specificity, non-invasive approach and possibility of repeated use to ensure real-time follow-up of the patient. However, liquid biopsy currently is not a routine laboratory test in clinical practice yet. Major developments are expected in the nearest years, including progress in technologies, software and quality control systems as well as diagnosis-based research to substantiate many promising clinical applications.

Acknowledgements

Our work has been supported by Riga Stradins University, Riga, Latvia; grant N.21-1/1/2019-4.

Conflict of interest

Editors have no conflicts of interest.

Author details

Ilze Strumfa^{1*} and Janis Gardovskis²

*Address all correspondence to: ilze.strumfa@rsu.lv

1 Department of Pathology, Riga Stradins University, Riga, Latvia

2 Department of Surgery, Riga Stradins University, Riga, Latvia

References

- [1] Bai Y, Zhao H. Liquid biopsy in tumors: Opportunities and challenges. *The Annals of Translational Medicine*. 2018;**6**(Suppl 1):S89. DOI: 10.21037/atm.2018.11.31
- [2] Kang HM, Kim GH, Jeon HK, Kim DH, Jeon TY, Park DY, et al. Circulating tumor cells detected by lab-on-a-disc: Role in early diagnosis of gastric cancer. *PLoS One*. 2017;**12**(6):e0180251
- [3] Guanciali Franchi P, Palka C, Morizio E, Sabbatinelli G, Alfonsi M, Fantasia D, et al. Sequential combined test, second trimester maternal serum markers, and circulating fetal cells to select women for invasive prenatal diagnosis. *PLoS One*. 2017;**12**(12):e0189235. DOI: 10.1371/journal.pone.0189235
- [4] Celec P, Vlkova B, Laukova L, Babickova J, Boor P. Cell-free DNA: The role in pathophysiology and as a biomarker in kidney diseases. *Expert Reviews in Molecular Medicine*. 2018;**20**:e1. DOI: 10.1017/erm.2017.12
- [5] Palmirotta R, Lovero D, Cafforio P, Felici C, Mannavola F, Pelle E, et al. Liquid biopsy of cancer: A multimodal diagnostic tool in clinical oncology. *Therapeutic Advances in Medical Oncology*. 2018;**10**:1758835918794630. DOI: 10.1177/1758835918794630
- [6] Merker JD, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review. *Journal of Clinical Oncology*. 2018;**36**(16):1631-1641. DOI: 10.1200/JCO.2017.76.8671
- [7] Gandara DR, Paul SM, Kowanetz M, Schleifman E, Zou W, Li Y, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nature Medicine*. 2018;**24**(9):1441-1448. DOI: 10.1038/s41591-018-0134-3
- [8] Odogwu L, Mathieu L, Goldberg KB, Blumenthal GM, Larkins E, Fiero MH, et al. FDA benefit-risk assessment of osimertinib for the treatment of metastatic non-small cell lung cancer harboring epidermal growth factor receptor T790M mutation. *The Oncologist*. 2018;**23**(3):353-359. DOI: 10.1634/theoncologist.2017-0425
- [9] Zhou J, Ma X, Bi F, Liu M. Clinical significance of circulating tumor cells in gastric cancer patients. *Oncotarget*. 2017;**8**(15):25713-25720. DOI: 10.18632/oncotarget.14879
- [10] Zheng L, Zou K, Yang C, Chen F, Guo T, Xiong B. Inflammation-based indexes and clinicopathologic features are strong predictive values of preoperative circulating tumor cell detection in gastric cancer patients. *Clinical and Translational Oncology*. 2017;**19**(9):1125-1132
- [11] MacFarlane LA, Murphy PR. MicroRNA: Biogenesis, function and role in cancer. *Current Genomics*. 2010;**11**(7):537-561
- [12] Hashemzadeh MR. Role of micro RNAs in stem cells, cardiac differentiation and cardiovascular diseases. *Gene Reports*. 2017;**8**:11-16

- [13] Yuan HL, Wang T, Zhang KH. MicroRNAs as potential biomarkers for diagnosis, therapy and prognosis of gastric cancer. *OncoTargets and Therapy*. 2018;**11**:3891-3900. DOI: 10.2147/OTT.S156921
- [14] Li S, Zhang T, Zhou X, Du Z, Chen F, Luo J, et al. The tumor suppressor role of miR-155-5p in gastric cancer. *Oncology Letters*. 2018;**16**(2):2709-2714. DOI: 10.3892/ol.2018.8932
- [15] Liu Q, Jiang J, Fu Y, Liu T, Yu Y, Zhang X. MiR-129-5p functions as a tumor suppressor in gastric cancer progression through targeting ADAM9. *Biomedicine and Pharmacotherapy*. 2018;**105**:420-427. DOI: 10.1016/j.biopha.2018.05.105
- [16] Hua K, Chen YT, Chen CF, Tang YS, Huang TT, Lin YC, et al. MicroRNA-23a/27a/24-2 cluster promotes gastric cancer cell proliferation synergistically. *Oncology Letters*. 2018;**16**(2):2319-2325. DOI: 10.3892/ol.2018.8924
- [17] Truong G, Guanzon D, Kinhall V, Elfeky O, Lai A, Longo S, et al. Oxygen tension regulates the miRNA profile and bioactivity of exosomes released from extravillous trophoblast cells—Liquid biopsies for monitoring complications of pregnancy. *PLoS One*. 2017;**12**(3):e0174514. DOI: 10.1371/journal.pone.0174514
- [18] Best MG, Wesseling P, Wurdinger T. Tumor-educated platelets as a noninvasive biomarker source for cancer detection and progression monitoring. *Cancer Research*. 2018;**78**(13):3407-3412. DOI: 10.1158/0008-5472.CAN-18-0887