Review

Detecting Blood-Based Biomarkers in Metastatic Breast Cancer: A Systematic Review of Their Current Status and Clinical Utility

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Abstract: Reviews on circulating biomarkers in breast cancer usually focus on one single biomarker or a selective group of biomarkers. An overview summarizing the discovery and evaluation of all blood-based biomarkers in metastatic breast cancer is lacking. This systematic review aims to identify the available evidence of known blood-based biomarkers in metastatic breast cancer, regarding their clinical utility and state-of-the-art position in the validation process. The initial search yielded 1078 original studies, of which 420 were assessed for eligibility. A total of 320 studies were included in the final synthesis. A Development, Evaluation and Application Chart (DEAC) of all biomarkers was developed. Most studies focus on identifying new biomarkers and search for relations between these biomarkers and traditional molecular characteristics. Biomarkers are usually investigated in only one study (68.8%). Only 9.8% of all biomarkers was investigated in more than five studies. Circulating tumor cells, gene expression within tumor cells and the concentration of secreted proteins are the most frequently investigated biomarkers in liquid biopsies. However, there is a lack of studies focusing on identifying the clinical utility of these biomarkers, by which the additional value still seems to be limited according to the investigated evidence.

Keywords: metastatic breast cancer; liquid biopsy; circulating biomarkers; blood-based biomarkers; circulating tumor cells (CTCs); utility; developmental stages; development evaluation and application chart (DEAC)

1. Introduction

1.1. Breast Cancer Survival

Globally, breast cancer is the most commonly diagnosed form of cancer among women. Clinical management has improved over the last years, and the development of genetic tests such as Mammaprint and OncoTypeDX have proven to guide treatment in early stage breast cancer. Although the current 5-year survival for primary breast cancer is relatively high (ranging from 80% to 92% in different populations) [1], survival rates decrease to less than 25% when the disease becomes metastatic [1,2]. The most important factor to increase survival for those suffering from metastatic breast cancer, is to prescribe a treatment that has the most likelihood of being effective, guided by the tumor cell characteristics [3,4]. To select the most effective treatment once the metastatic lesions have
been detected, it is essential to obtain accurate information on the characteristics of the tumor cells at the time therapy is to be initiated [5].

1.2. Detection and Treatment of Metastatic Lesions

Technical advances in the molecular characterization of cells has already lead to accurate predictions of survival and treatment efficacy. However, these molecular characterizations require high-quality biopsies, which cannot always be obtained from the primary tumor [6]. Alternatively, taking a biopsy of the metastatic lesion is either difficult or even impossible, for example, due to its location, or the inability to visualize that location with the currently used imaging techniques [6–8]. Furthermore, previous research has shown that molecular aberrations of the primary tumor may differ from that of the metastatic lesion and different metastatic lesions can have different characteristics [9]. Therefore, there remains a need for new tests which are sufficiently sensitive and reflect the composition of the tumor at all sites to guide treatment of metastatic disease.

1.3. The Use of Blood-Based Biomarkers

A possible way of enabling better treatment response monitoring or treatment guidance is the use of blood-based biomarkers or liquid biopsies [10]. A large number of single blood-based biomarkers can be distinguished in the blood, of which the most commonly known soluble proteins are Human Epidermal Growth Factor Receptor 2 (HER2), Cancer Antigen 15-3 (CA 15-3), Carcinoembryonic Antigen (CEA) and MUC1 [11]. Furthermore, all kinds of gene expression patterns or mutations can be extracted from circulating mRNA or circulating free DNA [8,12]. However, not only proteins or gene expression patterns yield prognostic or predictive information, even complete cells found in the blood—such as Circulating Tumor Cells (CTCs) or Cancer Associated Fibroblasts (CAF)—provide this type of information.

Although a range of different biomarkers is known, it is far more difficult to evaluate their usefulness for treatment targeting or prognosis of disease. It therefore is required to develop a classification, both to determine biomarkers with clinical utility and to prioritize future research. For clinical decision making, there are different ways of classifying diagnostic information [10]. Classifications focus, for example, on prognostic or predictive ability, or on a classification according to specific hallmarks of cancer [12].

1.4. Evidence on the Utility of Biomarkers

Up to now, the literature is not clear about the clinical utility of biomarkers in breast cancer. Several systematic reviews on blood-based biomarkers have been published yet [10,13]. However, these studies usually focus on one single biomarker or a selective group of biomarkers. These reviews are helpful to understand specific molecular pathways of oncogenesis, on specific prognostic information and all other outcomes they are related to, or on both.

An overview summarizing the discovery and evaluation of blood-based biomarkers for metastatic disease, in terms of their current status and future potential for clinical application, is still lacking. Therefore, this systematic review focusses on identifying known biomarkers, the available evidence regarding their clinical utility and exploring the current state-of-the-art in the validation process of all blood-based biomarkers in metastatic breast cancer. The review aims to identify a set of blood-based biomarkers that may have substantial future potential. Whereas it is common to focus on outcomes in terms of effectiveness, this review instead focusses on the developmental stage as the primary outcome measure of the included studies. First, all blood-based biomarkers will be identified and classified according to their developmental stage (e.g., from discovery to clinical utility). Second, the set of biomarkers with the highest future potential for clinical application will be identified by the number of studies that have been performed in each of the developmental stages.
1.5. Conclusions

The main aim of research on blood-based biomarkers in metastatic breast cancer is the identification of new biomarkers or relations of these biomarkers with other original molecular tumor characteristics. Especially gene expression within CTCs is investigated frequently. However, there still is a lack of studies identifying the clinical utility of these biomarkers. Thereby, the additional value for these biomarkers seems to be still limited according to the investigated evidence.

2. Results

2.1. Search Results

The initial search resulted in a total of 1249 studies from all databases searched. After screening all abstracts, 410 studies were further assessed for eligibility. During the assessment for eligibility, 91 studies were excluded. A total of 320 studies were included in this review. The full list of all studies that were included is presented in Appendix C. Most studies were excluded because the biomarkers investigated were not extracted from metastatic breast cancer patients \((n = 22; 24.4\%)\), because the blood used in the detection of the biomarker was non-human or was injected with a cell line that had just metastatic potential \((n = 19; 21.1\%)\) or because the study investigated multiple stages of breast cancer but had not reported conclusions for metastatic breast cancer separately \((n = 17; 18.9\%)\). The flow diagram of the search is presented in Figure 1.

2.2. Study Characteristics

For each study, the data were extracted and two classifications were made. First, the biomarkers were classified in one of the four general categories. Second, studies were classified in one of the pre-defined developmental stage categories, as defined in Figure 2. To illustrate the classification more clearly, citations of those studies which were classified as being in one of these phases are given in the right column of Figure 2.
2.3. Results According to Developmental Phase

Figure 3 presents the DEAC with the distribution of studies over developmental phases. From this figure it is apparent that most studies focused on the identification phase. This means that most studies focus on finding relationships between the concentration of the biomarker, in relation to a new or existing threshold and furthermore, try to evaluate this against an outcome measure in terms of survival (e.g., Overall Survival (OS), Progression Free Survival (PFS) or survival in months). This phase is split up over two sub phases, namely basic predictive and basic prognostic research. For predictive research, only the concentrations in a subgroup of metastatic breast cancer patients were reported. For prognostic research, these concentrations were linked to an outcome measure related to survival (OS or PFS).
2.4. Results per Biomarker

The general biomarker category in which most studies were performed on blood-based biomarkers in metastatic breast cancer, concerned whole cells in the blood ($n = 181; 56.6\%$). CTCs made up a large part of this. In $85.1\%$ ($n = 154$) of all included studies CTC enumeration was performed. In $42.5\%$ of all included studies ($n = 136$), also genetic profiling for these cells had been done. The markers most frequently investigated are presented in Table 1.

Only those biomarkers for which 5 or more studies have been performed are included in the table. This cut-off had been chosen because these markers represent the most frequently investigated biomarkers. The frequency by which biomarkers are investigated is presented in Table 2, which presents that only $9.8\%$ of all biomarkers is investigated in more than 5 studies. A detailed overview presenting the amount of studies performed for each single biomarker, including an overview of the amount of studies in each developmental stage is presented in Appendix D.
Table 1. Most frequently investigated biomarkers of all studies included.

| Biomarker   | Number of Articles | % of Included Studies | End Stage            | Number of Studies at End Stage |
|-------------|--------------------|-----------------------|----------------------|-------------------------------|
| ALDH1       | 5                  | 1.6%                  | Observational        | 1                             |
| CA15-3      | 51                 | 15.9%                 | Observational        | 6                             |
| CEA         | 19                 | 5.9%                  | Observational        | 1                             |
| CK19        | 6                  | 1.9%                  | Observational        | 1                             |
| CTC enumeration | 154             | 48.1%                 | Clinical trial       | 29                            |
| EGFR        | 15                 | 4.7%                  | Observational        | 6                             |
| ER          | 13                 | 4.1%                  | Basic prognostic     | 3                             |
| HER2        | 61                 | 19.1%                 | Observational        | 15                            |
| PIK3CA      | 13                 | 4.1%                  | Observational        | 1                             |
| PR          | 7                  | 2.2%                  | Basic prognostic     | 2                             |
| RASSF1A     | 6                  | 1.9%                  | Basic predictive     | 5                             |
| THBS-1      | 9                  | 2.8%                  | Observational        | 5                             |
| TP53        | 5                  | 1.6%                  | Basic prognostic     | 1                             |
| TWIST       | 7                  | 2.2%                  | Observational        | 2                             |
| VEGF        | 22                 | 6.9%                  | Observational        | 15                            |
| VEGFR       | 13                 | 4.1%                  | Observational        | 12                            |
| Vimentin    | 6                  | 1.9%                  | Basic prognostic     | 1                             |

* The abbreviations used are standard abbreviations. Corresponding gene identities encoding for these biomarkers are presented in Appendix D.

Table 2. Number of studies in which single biomarkers are investigated.

| Number of Studies that Investigated a Specific Biomarker | Frequency | % of All Included Studies |
|--------------------------------------------------------|-----------|---------------------------|
| 1                                                      | 190       | 68.8%                     |
| 2                                                      | 38        | 13.8%                     |
| 3                                                      | 12        | 4.3%                      |
| 4                                                      | 9         | 3.3%                      |
| 5–10                                                   | 19        | 6.9%                      |
| >10                                                    | 8         | 2.9%                      |

The percentages shown in Table 1 present the percentage of total studies that investigated that single marker. The second general biomarker category on which a relatively large amount of studies have been performed (n = 107; 33.4%) are proteins. Within this category most research has been focusing on 4 proteins, which are: CA15-3 (n = 22; 20.5%), soluble HER2 (n = 19; 17.8%), Vascular Endothelial Growth Factor (VEGF) (n = 18; 16.8%) and Vascular Endothelial Growth Factor Receptor (VEGFR) (n = 14; 13.1%). As discussed before, frequently studies are focusing on investigating multiple biomarkers instead of single biomarkers. As presented in Table 1, a total of 51 studies have investigated CA15-3. This means that also research which mainly focuses on one of the other biomarker categories investigates CA15-3. The same differences in the amount of studies performed were seen for HER2 and VEGF.

2.5. Results on the Number of Studies Performed

Summarized over all general biomarker categories, the total amount of studies included in the results synthesis is 320 as presented in Figure 2. In these studies a total of 275 single biomarkers have been investigated. The average number of studies performed on one single biomarker is 2.6 (range 1–154 studies). In Table 2 results are presented for frequency by which the study investigated a number of biomarkers. Table 2 shows that for 68.8% of all the biomarkers only one study has investigated that particular biomarker. For 13.8% of all biomarkers two studies have investigated that biomarker.
3. Discussion

In this paper we present a broad overview of research on blood-based biomarkers in metastatic breast cancer, performed since 2006. Of the included studies, most focused on detecting whole cells in the blood, with a focus on the enumeration or genetic characterization of circulating tumor cells. Considering the classification into developmental stages, the identification stage is the stage during which most research has been performed. Most studies focus on the identification phase, in which they investigate the ability to detect particular biomarkers in the blood and are trying to find connections between these concentrations and potential outcome measures in terms of survival. For proteins CA15-3, soluble HER2, VEGF and VEGFR have been investigated most frequently. However, for CTCs there have been clinical trials, but not for one of these proteins since 2006.

In terms of developmental stages, we expected that the amount of research performed would follow some kind of trend over time. It was expected that per biomarker there would be a substantial amount of studies focusing on the early developmental stages (technical validation), with decreasing numbers of studies the further the research for that particular biomarker proceeded in the developmental process. However, the DEAC shows that this trend does not exist for blood-based biomarkers in metastatic breast cancer. The DEAC shows that the number of studies performed increase until they reach the identification phase, and decrease afterwards. Therefore, it seems that the technical validation and clinical validation phase are currently less performed than research in the identification phase. Another observation from the DEAC is the low amount of research performed in the prognostic validation phase, suggesting this phase is not receiving sufficient attention. However, this may well be due to the fact that the initial search was limited to articles published since 2006, so that a limited amount of studies concerning some of the developmental phases were found. It might have been that specific phases which seemed to have had insufficient attention for several biomarkers were investigated before 2006. In addition, some information might have been missed, as publication bias may have occurred due to excluding non-English studies.

Furthermore, biomarker research may have been performed in a commercial setting or for stakeholders intent to guide internal research and development decisions. As such, selective reporting may occur by which not all findings might have been published. The same holds for studies with negative findings on (some subset) of investigated biomarkers, as it is known that such results are harder to publish than positive findings. As we did not investigate a single outcome measure, no standard methods are available to assess the ensuing risk of bias in our results. Even though the intention of reports might be to inform about recent developments, other stakeholders might use this information differently. Therefore, it seems valuable for future research to be able to have access to all information that was, or can possibly be extracted from the blood samples. Future research should pay attention to selective reporting before publishing, or ensure that samples are publicly available via biobanks.

4. Materials and Methods

This systematic review of blood-based biomarkers in metastatic breast cancer was performed according to the PRISMA guidelines [22]. A review protocol was used and is presented in Appendix A. This review was not registered in the PROSPERO database. All types of studies were included in the initial review, as the aim of this review is to identify the best available evidence exploring the position in the development process of all blood-based biomarkers in metastatic breast cancer. Since all types of primary research studies were included, it was not required that the intervention, control or specific outcome measure was reported in the initial search. Therefore, no specific study characteristics or PICO-statement for inclusion criteria was used. The only restriction applied to the search concerned a time constraint, as studies published since 1 January 2006 were included. Databases that were searched are PubMed, Scopus and OVID. Additionally, articles found by cross-referencing or hand search were included in the initial search. The initial search was performed in June 2016 and updated on 1 December 2016. The detailed search terms applied are presented in Appendix B.
After the initial search and removal of duplicate papers, abstracts were scanned for relevance. Abstracts of articles that either did not present non-primary research data or concerned topics not of interest here (such as, other cancer types, only other stages of breast cancer, and non-blood-based biomarkers—e.g., biomarkers that can be found in other body fluids) were excluded from the full-text review. All abstracts were processed by one reviewer (A. M. Sofie Berghuis) and were discussed with a second reviewer (Hendrik Koffijberg) if necessary.

Full texts of all included papers were assessed for eligibility by one reviewer (A. M. Sofie Berghuis). All studies were then categorized according to the 10 pre-defined developmental stage and per general biomarker type. Four general developmental stages were identified, namely technical validation, identification, clinical validation and clinical utility. A full description of all pre-defined developmental stages is presented in Figure 1. Data was then classified in four general types of biomarkers, namely cells, proteins, circulating DNA and circulating RNA. Final classification of studies was discussed with a second reviewer (Hendrik Koffijberg) if classification in either one of the categories was unclear to the first reviewer (A. M. Sofie Berghuis). For studies on which there was no consensus between these two reviewers, a third reviewer reclassified the study (Maarten J. IJzerman).

4.1. Article Processing

Quantitative and qualitative data was manually extracted from the included studies and structured in Excel (version 2013) in pre-defined and labeled columns. The following information was extracted from all the included studies:

- General biomarker classification (classification in one of the four categories: cells, proteins, circulating DNA or circulating RNA)
- Developmental stage (classification according to the stages and general descriptions of these stages shown in Figure 1)
- Specific biomarker name
- Type of test used to quantify or detect biomarker (e.g., ELISA, CellSearch, etc.)
- Whether—and if so, which—survival data was presented (Overall Survival, Progression Free Survival, survival in months)

Given the focus on the translation of biomarkers to clinical practice, the results of all included studies were summarized according to the number of studies performed per developmental stage for each general biomarker category. Results for all single biomarkers were summarized per general biomarker category as studies might investigate more than one biomarker. For all single biomarkers it was determined how many studies investigated that biomarker and in which stage of translation the biomarker was identified. For each general biomarker category it was investigated how many studies presented results on the full range of single biomarkers found.

4.2. Synthesis of Results

Results were presented in a Development, Evaluation and Application Chart (DEAC) that was developed specifically for this review. This figure gives a broad overview of the development of biomarkers in each of the predefined stages of clinical translation. Specifically, the figure shows four bar diagrams above each other, one diagram for each of the general biomarker categories. Each vertical bar, per diagram, represents a developmental stage. The bars are displayed to represent the different stages in the translation, starting with the most basic (developmental) research on the left side and more advanced (evaluation) research (such as clinical trials or health economic evaluations) presented on the right side. The height of the bars reflects the number of included studies. This figure therefore gives an overview of the number of studies published on each of the general biomarker categories according to the developmental stage timeline.
5. Conclusions

Since 2006, a substantial amount of research has been done to investigate the potential role of blood-based biomarkers in metastatic breast cancer. There seems to be a focus on research toward the use of CTCs, as most studies investigate these, whether in combination with other markers or as a single marker. The current emphasis of investigating these biomarkers seems to be on developing new techniques or finding new biomarkers that might have predictive or prognostic value, as most studies focus on the identification phase. There is a lack of studies focusing on clinical utility of these biomarkers. This might be because these studies have not yet been performed or suffer from publication bias. However, the lack of studies investigating the utility of blood-based biomarkers causes the additional value in terms of clinical utility, health outcomes or health care efficiency to still be limited according to the investigated evidence.

Author Contributions: The initial search was performed by A. M. Sofie Berghuis. Classification into developmental stages of the articles was discussed with Hendrik Koffijberg and Maarten J. IJzerman. Classification of the general biomarker categories was discussed with Jai Prakash and Leon W. M. M. Terstappen. All authors were involved in writing and revising the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

| Abbreviation | Description                          |
|--------------|--------------------------------------|
| CA15-3       | Cancer Antigen 15-3                   |
| CTC          | Circulating Tumor Cell               |
| DEAC         | Development, Evaluation and Application Chart |
| PFS          | Progression Free Survival            |
| HER2         | Human Epidermal Growth Factor Receptor 2 |
| OS           | Overall Survival                     |
| VEGF         | Vascular Endothelial Growth Factor    |
| VEGFR        | Vascular Endothelial Growth Factor Receptor |

Appendix A. Review Protocol

The steps described in Figure A1 were used as a review protocol.

In the abstract, screening the abstracts were only excluded when they explicitly presented that they contained information on one of the five exclusion criteria. If it was doubted whether results for blood-based biomarkers could have been presented in the text due to vague descriptions, the article was included for full text review. An example of this is the use of “Advanced breast cancer” as study population. For advanced often stage IIIB/IIIC and stage IV is meant. However, several abstracts did not present which stages they exactly investigated. These abstracts therefore were included.

Non-primary research that was excluded from further synthesis was saved in a separate folder, so these can be easily accessed after the complete search. Therefore, comparisons between the results found and the results that were already presented could easily be made.
Appendix B. Search Terms

Below the full electronic search strategy is presented, i updated until 1 December 2016.

Appendix B.1. Final Search Term PubMed (n = 994)

(((Metastatic[tiab] OR Advanced [tiab] OR Late stage[tiab] OR Malign*[tiab] OR Stage IV[tiab] OR Secondary[tiab]) AND (Breast cancer[majr] OR Breast neoplasm[majr] OR Breast carcinoma[majr] OR Mamma carcinoma[majr] OR Breast tumor[majr] OR Breast tumour[majr] OR “breast neoplasms”[Mesh])) AND ((Blood based[tw] OR Circulating[tw] OR Plasma[tw] OR Liquid[tw] OR Serum[tw] OR Serum based[tw] OR Extracellular[tw]) AND (blood[tw] AND (Marker*[tw] OR Biomarker*[tw] OR Biops*[tw] OR Tumor Cell*[tw] OR Tumor micro particle*[tw] OR Tumor particle*[tw] OR Tumor vesicle*[tw] OR Tumour Cell*[tw] OR Tumour micro particle*[tw] OR Tumour particle*[tw] OR Tumour vesicle*[tw] OR Tumor marker*[tw] OR Tumour marker*[tw] OR Tumor cell cluster*[tw] OR Tumour cell cluster*))))

Appendix B.2. Final Search Term Scopus (n = 6)

(((ALL(blood based OR circulating OR plasma OR liquid OR serum OR serum based OR extracellular)) AND (ALL (marker OR biomarker OR biops OR tumor cell OR tumor micro particle OR tumor particle OR tumor vesicle OR tumour cell OR tumour micro particle OR tumour particle OR tumour vesicle OR tumor marker OR tumour marker OR tumor cell cluster OR tumour cell cluster)) AND (ALL (blood))) AND ((ABS (metastatic OR advanced OR late stage OR malign OR stage iv OR secondary)) AND (ALL (breast cancer OR breast neoplasm OR breast carcinoma OR mamma carcinoma OR breast tumor OR breast tumour)))

Figure A1. Review Protocol.
Appendix B.3. Final Search Terms OVID (n = 90)

(((ALL (blood based OR circulating OR plasma OR liquid OR serum OR serum based OR extracellular))
AND (ALL (marker OR biomarker OR biops OR tumor cell OR tumor micro particle OR tumor particle
OR tumor vesicle OR tumour cell OR tumour micro particle OR tumour particle OR tumour vesicle OR tumor marker OR tumour marker OR tumor cell cluster OR tumour cell cluster))) AND (ALL (blood)))
AND ((ABS (metastatic OR advanced OR late stage OR malign OR stage iv OR secondary)) AND (ALL
(breast cancer OR breast neoplasm OR breast carcinoma OR mamma carcinoma OR breast tumor OR
breast tumour))))

Appendix B.4. Final Search Terms Medline (n = 159)

(((TI = (metastatic OR advanced OR late stage OR malign OR stage iv OR secondary)) AND (TI =
(breast cancer OR breast neoplasm OR breast carcinoma OR mamma carcinoma OR breast tumor OR
breast tumour))) AND ((TI = (blood based OR circulating OR plasma OR liquid OR serum OR serum
based OR extracellular)) AND (TI = (marker OR biomarker OR biops OR tumor cell OR tumor micro
particle OR tumor particle OR tumor vesicle OR tumour cell OR tumour micro particle OR tumour
particle OR tumour vesicle OR tumor marker OR tumour marker OR tumor cell cluster OR tumour
cell cluster)))) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)
Indexes = SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan = 2006–2016

Appendix C. Overview of All Included Studies

1. Zhang SH, Li L, Wang T, Bian L, Hu HX, Xu CH, et al. Real-time HER2 status detected
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HER2-positive metastatic breast cancer patients. BMC cancer. 2016;16.
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breast cancer patients. BMC cancer. 2016;16.
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Appendix D. Detailed Overview of All Biomarkers per Developmental Stage

For all biomarkers an overview was created of the number of studies that investigated the biomarker and the translational stages in which these studies have been performed. Some studies focused on biomarkers from multiple categories (e.g., they focused on cells and proteins at the same time). All biomarkers investigated in these studies were categorized according to their main research subject. If the article for example mainly studied CTCs but also measured the concentration of CA15-3, then this study was classified in the cells category. In the following sections an overview of all biomarkers that were classified in the four general biomarker categories is presented.

Appendix D.1. Cells

Several types of research on cells as blood-based biomarkers have been performed. Studies enumerated cells, investigated the expression of proteins on their membrane or investigated the expression of genes that extracted from DNA or mRNA of the cell. The first type of studies are those investigating the enumeration of whole cells or cell clusters. All cells or cell clusters that have been investigated are presented in Table A1.

The second type of studies that investigated cells are those studies that investigate the proteins that are expressed on the membranes of the cells. Table A2 presents the proteins that have been investigated on the membranes of cells. The fourth column “Gene” presents the abbreviations of the genes that encode for these proteins. The fifth column “Gene ID” presents the gene ID of the gene that encodes for the membrane protein.

The third type of studies included in the cells category presented research on the gene expression within these cells. Table A3 presents the genes have been investigated, after DNA or mRNA was extracted from cells.
Studies that mainly focus on cells have also investigated a couple of proteins parallel to their research on the enumeration of cells, membrane expression of proteins on those cells or gene expression within those cells. Table A4 presents the proteins that have been investigated in studies that mainly focused on cells.

Besides the above presented blood-based biomarkers, in one study microRNA 10b had been investigated in parallel with research on CTCs (basic predictive research). In five other studies DNA from cells was extracted and whole genome amplification was performed (all 5 studies were categorized as basic predictive research).

Appendix D.2. Proteins

Several types of proteins have been investigated. Table A5 presents an overview of all proteins that have been investigated in studies which mainly focus on proteins as blood-based biomarkers. In these studies the main research aim is on proteins as blood-based biomarkers. However, also a couple of other biomarkers have been investigated. In two studies they simultaneously investigated the enumeration of CTCs. In eight studies they looked at gene expression patterns within DNA or mRNA. The genes investigated in these studies were OPG, RANKL, eNOS, and THBS-1.

Appendix D.3. DNA

Table A6 presents all genes that have been investigated in studies that mainly focus on circulating DNA.

Besides DNA investigated, several articles investigated proteins or cells in parallel. Two studies enumerated CTCs in parallel (basic prognostic research). In 13 studies proteins were investigated in parallel. Seven studies focused on CA15-3, five on CEA and 1 on the plasminogen activator.

Appendix D.4. RNA

Table A7 presents all circulating micro RNAs that have been studied.

Studies that mainly focused on microRNAs investigated the enumeration of CTCs in parallel in two studies, and the concentration of secreted Mammaglobin in one study.
Table A1. Translational stages of research on the enumeration of whole cells or cell clusters.

| Biomarker Abbreviation | Biomarker | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|------------------------|-----------|--------------------------|----------------|-----------------|----------------------|--------------------------|--------------------------|---------------------|----------------------|-------------------------|
| CAMLs                  | Cancer associated macrophage like cells | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| CAFs                   | Cancer associated fibroblasts | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Monocyte CD63          | Type of white blood cell CD63 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Monocyte CD64          | Type of white blood cell CD64 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| CECs                   | Circulating epithelial cells | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| CEPs                   | Circulating epithelial progenitor cells | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| CTEC                   | Circulating epithelial tumor cells | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| aCTCs                  | Apoptotic circulating tumor cells | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| CSCs                   | Cancer Stem Cells | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| CTC                    | Circulating tumor cells | 156 | 4 | 19 | 20 | 46 | 35 | 1 | 29 | 2 |
| CTC Cluster            | Circulating tumor cell clusters | 3 | 1 | 1 | 1 | 1 | 1 | 1 |
| NKs                    | Natural Killer Cells | 2 | 2 | 2 | 2 | 2 | 2 | 2 |

Table A2. Translational stages of research on the membrane expression of proteins.

| Biomarker Abbreviation | Biomarker | Cell * | Gene | Gene ID | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|------------------------|-----------|--------|------|---------|--------------------------|----------------|-----------------|----------------------|--------------------------|--------------------------|---------------------|----------------------|-------------------------|
| Akt2                   | AKT serine-threonine kinase 2 | CTC | AKT2 | 208 | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| CD133                  | Prominin 1 | CTC | PROM1 | 8842 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| CD44                   | CD44 molecule | CTC | CD44 | 960 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ER                     | Estrogen | CTC | ESR1 | 2099 | 13 | 2 | 1 | 6 | 3 | 3 | 3 | 3 | 3 |
| Fibronectin            | Fibronectin | CTC | FN1 | 2335 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| HER 2                  | Human epidermal growth factor receptor 2 | CTC | ERBB2 | 2064 | 25 | 1 | 1 | 3 | 8 | 4 | 0 | 8 | 8 |
| N-Cadherin             | Cadherin 2 | CTC | CDH2 | 1000 | 3 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| PR                    | Progesteron | CTC | PGR | 5241 | 7 | 1 | 4 | 2 | 2 | 2 | 2 | 2 | 2 |
| VEGF                   | Vascular endothelial growth factor | CTC | VEGFA | 7422 | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| VEGFR2                 | Vascular endothelial growth factor receptor 2 | CTC | KDR | 3791 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Vimentin               | Vimentin | CTC | VIM | 7431 | 6 | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| CD24                   | CD24 molecule | Granulocytes | CD24 | 10033941 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| TLR2                   | Toll Like Receptor 2 | Lymphocytes | TLR2 | 7097 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| TLR4                   | Toll Like Receptor 4 | Lymphocytes | TLR4 | 7099 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

* Cells given in this column represent the cells in which these biomarkers were found.
Table A3. Translational stages of research on gene expression within cell DNA or mRNA.

| Biomarker Abbreviation | Biomarker | Cell * | Gene | Gene ID | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|------------------------|-----------|--------|------|--------|--------------------------|----------------|------------------|--------------------|--------------------------|--------------------------|----------------------|-------------------|-------------------------|
| CD34                   | CD34 Molecule | CSCs   | CD34 | 947    | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| Nanog                  | NANOG     | CSCs   | NANOG| 79923  | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| Nestin                 | Nestin    | CSCs   | NES  | 10763  | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| Oct3/4                 | POL3F1    | CSCs   | POL3F1| 5460   | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| Sox2                   | SOX2      | CSCs   | SOX2 | 6657   | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| ACTA1                  | ACTA1     | CTC    | ACTA1| 58     | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| AGR2                   | AGR2      | CTC    | AGR2 | 10551  | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| ALDH1                  | ALDH1A1   | CTC    | ALDH1A1| 216    | 5                        | 1              | 1                | 2                  | 1                        |                          |                      |                   |                         |
| AURKA                  | AURKA     | CTC    | AURKA| 6790   | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| BCL2                   | BCL2      | CTC    | BCL2 | 596    | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| BIRC5                  | BIRC5     | CTC    | BIRC5| 332    | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| CCND1                  | CCND1     | CTC    | CCND1| 595    | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| CDC6                   | CDC6      | CTC    | CDC6 | 990    | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| CENPF                  | CENPF     | CTC    | CENPF| 1063   | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| CEP55                  | CEP55     | CTC    | CEP55| 55165  | 2                        | 1              | 1                | 1                  |                           |                          |                      |                   |                         |
| CK19                   | KRT19     | CTC    | KRT19| 3880   | 6                        | 4              | 1                | 1                  |                           |                          |                      |                   |                         |
| CK8                    | KRT8      | CTC    | KRT8 | 3856   | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| CRABP2                 | CRABP2    | CTC    | CRABP2| 1382   | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| CSTI6 promoter         | CSTI6     | CTC    | CSTI6| 1474   | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| CXCL14                 | CXCL14    | CTC    | CXCL14| 9547   | 2                        | 2              |                   |                    |                           |                          |                      |                   |                         |
| CXXC5                  | CXXC5     | CTC    | CXXC5| 51523  | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| DTX3                   | DTX3      | CTC    | DTX3 | 196403 | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| DUSP4                  | DUSP4     | CTC    | DUSP4| 1846   | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| EEF1A2                 | EEF1A2    | CTC    | EEF1A2| 1917   | 2                        | 2              |                   |                    |                           |                          |                      |                   |                         |
| EGFR                   | EGFR      | CTC    | EGFR | 1956   | 6                        | 1              | 2                | 1                  | 2                        |                          |                      |                   |                         |
| ERBB3                  | ERBB3     | CTC    | ERBB3| 2065   | 2                        | 1              | 1                | 1                  |                           |                          |                      |                   |                         |
| ERBB4                  | ERBB4     | CTC    | ERBB4| 2066   | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| ERCC1                  | ERCC1     | CTC    | ERCC1| 2067   | 1                        | 1              |                   |                    |                           |                          |                      |                   |                         |
| Biomarker Abbreviation | Biomarker | Cell * | Gene | Gene ID | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|------------------------|-----------|--------|------|--------|--------------------------|---------------|-----------------|----------------------|--------------------------|--------------------------|----------------------|---------------------|----------------------|
| ESR1                   | Estrogen Receptor 1 | CTC | ESR1 | 2099 | 3            | 1              | 2               |                      |                          |                          |                      |                     |                     |
| FGFR4                  | Fibroblast growth factor receptor 4 | CTC | FGFR4 | 2264 | 2            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| FKBPI0                | FK506 binding protein 10 | CTC | FKBPI0 | 60681 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| FOX A1                | Forkhead box A1 | CTC | FOX A1 | 3169 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| FOXC1                 | Forkhead box C1 | CTC | FOXC1 | 2296 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| GAPDH                 | Glyceraldehyde-3-phosphate dehydrogenase | CTC | GAPDH | 2907 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| HER2                  | Human epidermal growth factor receptor 2 | CTC | EBB2 | 2064 | 17           | 3              | 2               | 9                    | 1                        | 1                        |                      |                     |                     |
| HIF-1a                | hypoxia inducible factor-1 α | CTC | HIF1A | 3091 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| IGFBP2                | Insulin like growth factor binding protein 2 | CTC | IGFBP2 | 3485 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| IGFBP4                | Insulin like growth factor binding protein 4 | CTC | IGFBP4 | 3487 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| IL17B                 | Interleukin 17 receptor B | CTC | IL17B | 35940 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| ITGA6                 | Integrin subunit α | CTC | ITGA6 | 3655 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| Ki67                  | (proliferation marker) | CTC | MK67 | 4288 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| KRT14                 | Keratin 14 | CTC | KRT14 | 3861 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| KRT17                 | Keratin 17 | CTC | KRT17 | 3872 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| KRT19                 | Keratin 19 | CTC | KRT19 | 3880 | 2            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| KRT20                 | Keratin type 1 cytoskeletal 20 | CTC | KRT20 | 34474 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| KRT1                  | Keratin 7 | CTC | KRT7 | 3855 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| KRT81                 | Keratin 81 | CTC | KRT81 | 3887 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| LAD1                  | Ladinin 1 | CTC | LAD1 | 3898 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| Mamma-globin          | Secretoglobulin family 2a member 2 | CTC | SCGB2A2 | 4250 | 3            | 1              | 2               |                      |                          |                          |                      |                     |                     |
| MELK                  | Maternal embryonic leucine zipper kinase | CTC | MELK | 9833 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| MUC1                  | CAR1-3 | CTC | MUC1 | 4382 | 5            | 1              | 1               | 2                    |                          |                          |                      |                     |                     |
| MYBL2                 | MYB proto-oncogene like 2 | CTC | MYBL2 | 4605 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| NDC80                 | NDC80 Kinetochore complex component | CTC | NDC80 | 10403 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| NUF2                  | NUF2, NDC80 Kinetochore complex component | CTC | NUF2 | 83540 | 1            | 1              | 1               |                      |                          |                          |                      |                     |                     |
| Biomarker Abbreviation | Biomarker Description | Cell * | Gene | Gene ID | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|------------------------|-----------------------|--------|------|--------|--------------------------|----------------|-----------------|--------------------|-------------------------|--------------------------|------------------------|----------------------|---------------------|
| PIK3CA                 | Phosphatidylinositol-4,5-bisphosphate 3 kinase catalytic subunit α | CTC    | PIK3CA | 5290   | 7                        | 1              | 1               | 4                  | 1                       | 1                       | 1                      | 2                   | 1                   |
| PIP                    | Prolactin induced protein | CTC    | PIP   | 5304   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| PKF3                   | Plakophilin 3          | CTC    | PKF3  | 11187  | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| PTPRK                  | Protein tyrosine phosphatase, receptor type K | CTC    | PTPRK | 5796   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| PTRF                   | Polymerase I and transcript release factor | CTC    | PTRF  | 284119 | 2                        | 2              | 1               |                    |                         |                          |                        |                      |                     |
| PTTG1                  | Pituitary tumor transforming 1 | CTC    | PTTG1 | 9232   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| RRM2                   | Ribonucleotide reductase regulatory subunit M2 | CTC    | RRM2  | 6241   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| S100A7                 | S100 calcium binding protein A7 | CTC    | S100A7 | 6278  | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| SCGB1D2                | Secretoglobin family 1D member 2 | CTC    | SCGB1D2 | 10647 | 1                        | 1              | 2               |                    |                         |                          |                        |                      |                     |
| SLLG                   | Snail family transcriptional repressor 2 | CTC    | SNAI2 | 6591   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| SNAI1L                 | Snail family transcriptional repressor 1 | CTC    | SNAI1 | 6615   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| SPDEF                  | SAM Pointed domain containing ETS transcription factor | CTC    | SPDEF | 25803  | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| TFF3                   | Trefoil factor 3       | CTC    | TFF3  | 7033   | 2                        | 2              | 1               |                    |                         |                          |                        |                      |                     |
| TMEM45B                | Transmembrane protein 45B | CTC    | TMEM45B | 120224 | 1                      | 1              | 1               |                    |                         |                          |                        |                      |                     |
| TSPAN13                | Tetraspanin 13        | CTC    | TSPAN13 | 27075 | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| TWIST1                 | TWIST                 | CTC    | TWIST1 | 7291   | 7                        | 3              | 2               | 2                  | 2                       |                          |                        |                      |                     |
| TYMS                   | Thymidylate synthetase | CTC    | TYMS  | 7298   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| UBE2C                  | Ubiquitin conjugating enzyme E2 C | CTC    | UBE2C | 11065  | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| UBE2T                  | Ubiquitin conjugating enzyme E2 T | CTC    | UBE2T | 29089  | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| uPAR                   | Tyrosine plasminogen activator receptor | CTC    | PLA1U | 5328   | 2                        | 2              | 1               |                    |                         |                          |                        |                      |                     |
| TP53                   | Tumor Protein P53      | CTC    | TP53  | 7157   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| MRP1                   | ATP binding cassette subfamily C member 1 | CTC    | ABCC1 | 4363   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| MRP2                   | ATP binding cassette subfamily C member 2 | CTC    | ABCC2 | 1244   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| CK18                   | Cytokeratin 18        | CTC    | KRT18 | 3875   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| TFF1                   | Trefoil factor 1      | CTC    | TFF1  | 7031   | 2                        | 2              | 1               |                    |                         |                          |                        |                      |                     |
| RMS1                   | RMS1 ribosome biogenesis factor | CTC    | RMS1  | 9789   | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |
| SOX17                  | SOX Box 17            | CTC    | SOX17 | 64321  | 1                        | 1              | 1               |                    |                         |                          |                        |                      |                     |

* Cells given in this column represent the cells in which these biomarkers were found.
Table A4. Translational stages of research on proteins investigated in parallel in studies which mainly focus on cells.

| Biomarker Abbreviation | Biomarker | Membrane or Secreted Protein | Gene | Gene ID | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|------------------------|-----------|------------------------------|------|---------|--------------------------|---------------|------------------|----------------------|--------------------------|--------------------------|----------------------|------------------------|--------------------------|
| HER2                   | Human epidermal growth factor receptor 2 | Membrane | MUC1 | 4582    | 3                         | 2              | 1                |                      |                          |                          |                      |                        |                          |
| pFAK                  | phosphorylated-focal adhesion kinase    | Membrane | PTK2 | 5747    | 1                         |                |                  |                      |                          |                          |                      |                        |                          |
| CAIX                   | Carbonic anhydrase IX                   | Secreted | CAIX | 768     | 1                         |                |                  |                      |                          |                          |                      |                        |                          |
| CEA                   | Carcinoembryonic antigen related cell adhesion molecule | Secreted | CEACAM5 | 1048 | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| CXCL1                 | chemokine (C-X-C Motif) Ligand-1        | Secreted | CXCL1 | 2919 | 1 | | | | | | | | |
| Fibrinogen            | Fibrinogen                             | Secreted | FGA | 2243 | 1 | 1 | | | | | | | |
| LDH A                 | Lactate dehydrogenase                   | Secreted | LDH A | 3939 | 2 | 1 | 1 | | | | | | |
| MIB                 | Cytokeratin 18 fragments                | Secreted | KRT18 | 3855 | 3 | 1 | | | | | | | |
| PS5                  | Tumor Protein PS5                       | Secreted | TP5 | 7157 | 1 | | | | | | | | |
| TGFB-β                 | Transcription Growth Factor Beta 1      | Secreted | TGFB1 | 7040 | 1 | | | | | | | | |
| TIMP-1                 | TIMP metalloproteinase inhibitor 1      | Secreted | TIMP1 | 7078 | 1 | | | | | | | | |
| Bcl-2              | BCL2 apoptosis regulator                | Secreted | BCL2 | 596 | 1 | | | | | | | | |

Table A5. Translational stages of proteins.

| Biomarker Abbreviation | Biomarker | Type of Biomarker | Gene | Gene ID | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|------------------------|-----------|-------------------|------|---------|--------------------------|---------------|------------------|----------------------|--------------------------|--------------------------|----------------------|------------------------|--------------------------|
| EGFR                   | Epidermal growth factor receptor       | Membrane | EGFR | 1956 | 9 | 1 | | | | | | | |
| ENDOTR                 | Marrow Receptor, C type 2 (Endo 180)   | Membrane | MRC2 | 9912 | 1 | | | | | | | | |
| Endoglin               | Glycoprotein co-receptor for peptides in the TGF family | Membrane | ENG | 2022 | 1 | | | | | | | | |
| E-selectin            | Selectin E (SELE)                      | Membrane | SELE | 6401 | 2 | 1 | | | | | | | |
| HER2                  | Soluble Human epidermal growth factor receptor 2 | Membrane | EPRR2 | 2064 | 19 | 5 | 8 | 6 | | | | |
| Jagged 1              | Jagged 1                               | Membrane | NOTCH1 | 4851 | 1 | | | | | | | | |
| PDGFR-α                | Platelet derived growth factor receptor α | Membrane | PDGFRα | 5156 | 1 | | | | | | | | |
| RAGE                   | Soluble receptor for advanced glycation end products (sRAGE) | Membrane | AGER | 177 | 1 | | | | | | | | |
| RCF                    | Red Cell Folate                        | Membrane | FOLR1 | 2348 | 1 | | | | | | | | |
Table A5. Cont.

| Biomarker Abbreviation | Biomarker | Type of Biomarker | Gene | Gene ID | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|------------------------|-----------|-------------------|------|---------|--------------------------|----------------|-------------------|------------------------|--------------------------|--------------------------|----------------------|------------------------|--------------------------|
| sKIT                   | Stem cell factor receptor/KIT proto-oncogene receptor tyrosine kinase | Membrane | KIT | 3815 | 1 | | | | | 1 | | | |
| VEGFR-1                | Vascular Endothelial Growth Factor Receptor 1 | Membrane | FLT1 | 2321 | 1 | | | | | 1 | | |
| VEGFR-1                | Vascular Endothelial Growth Factor Receptor 1 | Membrane | FLT1 | 2321 | 2 | | | | | 2 | | |
| VEGFR-1                | Vascular Endothelial Growth Factor Receptor 1 | Membrane | FLT1 | 2321 | 2 | | | | | 2 | | |
| VEGFR-2                | Vascular Endothelial Growth Factor Receptor 2 | Membrane | KDR | 3791 | 1 | | | | | 1 | | |
| VEGFR-2                | Vascular Endothelial Growth Factor Receptor 2 | Membrane | KDR | 3791 | 3 | | | | | 3 | | |
| VEGFR-2                | Vascular Endothelial Growth Factor Receptor 2 | Membrane | KDR | 3791 | 4 | | | | | 1 | | |
| VEGFR-3                | Vascular endothelial growth factor receptor 3 | Membrane | FLT4 | 2324 | 1 | | | | | 1 | | | |
| a2-HS-glyco-protein    | Fetuin-A | Secreted | AHSG | 397 | 1 | | | | | 1 | | | |
| Activin A              | Inhibin beta A subunit | Secreted | INHBA | 3624 | 1 | | | | | 1 | | | |
| ALP                    | serum Alkaline Phosphatase | Secreted | ALPL | 249 | 4 | | | | | 1 | | |
| bFGF                   | Fibroblast growth factor 2 | Secreted | FGF2 | 2247 | 1 | | | | | 1 | | | |
| big ET-1               | big endothelin 1-growth factor | Secreted | EDN1 | 1906 | 1 | | | | | 1 | | | |
| CA19-9                 | Carbohydrate antigen | Secreted | 2 | 1 | | | | | | | | | |
| CAIX                   | Carbonic anhydrase IX | Secreted | CA9 | 768 | 2 | | | | | 1 | | |
| CEA                    | Carcinoembryonic antigen (CEA) | Secreted | CEACAM5 | 1084 | 8 | | | | | 1 | | |
| CML                    | Carboxymethyllysine | Secreted | BCR | 613 | 1 | | | | | 1 | | | |
| CRP                    | C-reactive protein | Secreted | CRP | 1401 | 1 | | | | | 1 | | | |
| CTX                    | C-terminal telopeptide | secreted | CYP27A1 | 1593 | 3 | | | | | 1 | | |
| CYFRA21-1              | Cytokeratin 19 Fragment | Secreted | KRT19 | 3880 | 1 | | | | | 1 | | | |
| Cyst C                 | inhibitor of IL-6 | Secreted | 1 | 1 | | | | | | | | | |
| DKK-1                  | Dickkopf-1 (DKK1) | Secreted | DKK1 | 25943 | 2 | | | | | 1 | | |
| ENO1 ABS               | Antibodies against human a enolase | Secreted | ENO1 | 2023 | 1 | | | | | 1 | | | |
| ES                     | Endostatin | Secreted | CD133 | 80781 | 1 | | | | | 1 | | | |
| FASN                   | Fatty acid synthase | Secreted | FASN | 2194 | 2 | | | | | 1 | | | |
Table A5. Cont.

| Biomarker Abbreviation | Biomarker | Type of Biomarker | Gene | Gene ID | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|------------------------|-----------|-------------------|------|---------|-------------------------|----------------|-----------------|----------------------|---------------------------|--------------------------|------------------------|------------------------|--------------------------|
| FSIP1                  | Fibrous sheath interacting protein | Secreted | FSIP1 | 161835 | 2 | 2 | | | | | | | |
| Galectin-1             | Galectin-1 | Secreted | LGALS1 | 3956 | 1 | 1 | | | | | | | |
| Galectin-8             | Galectin-8 | Secreted | LGALS8 | 3964 | 1 | 1 | | | | | | | |
| GASP-1                 | G-protein coupled receptor associated sorting protein 1 | Secreted | GPRASP1 | 9737 | 1 | 1 | | | | | | | |
| G-CSF                  | Granulocyte colony stimulating factor | Secreted | CSF3R | 1441 | 1 | 1 | | | | | | | |
| GDF15                  | Growth factor differentiation factor 15 | Secreted | GDF15 | 9518 | 1 | 1 | | | | | | | |
| GGT                    | Gamma-Glutamyltransferase | Secreted | GGT1 | 2678 | 1 | 1 | | | | | | | |
| HMGB1                  | soluble high-mobility group box 1 (HMGB1) | Secreted | HMGB1 | 3146 | 1 | 1 | | | | | | | |
| HSP70                  | Heat shock protein 70 | Secreted | HSP54 | 3308 | 1 | | | | | | | | |
| IFN-γ                  | Interferon gamma | Secreted | IFNG | 3458 | 1 | | | | | | | | |
| IL-10                  | Interleukin 10 | Secreted | IL10 | 3586 | 1 | 1 | | | | | | | |
| IL-18                  | Interleukin 18 | Secreted | IL18 | 3606 | 1 | 1 | | | | | | | |
| IL-2                   | Interleukin 2 | Secreted | IL2 | 3558 | 1 | | | | | | | | |
| IL-4                   | Interleukin 4 | Secreted | IL4 | 3505 | 1 | 1 | | | | | | | |
| IL-6                   | Interleukin 6 | Secreted | IL6 | 3569 | 4 | 2 | 2 | | | | | | |
| IL-7                   | Interleukin 7 | Secreted | IL7 | 3574 | 1 | 1 | | | | | | | |
| IL-8                   | CXC motif chemokine ligand 8 | Secreted | CXCL8 | 3576 | 1 | | | | | | | | |
| LDHA                   | Lactate dehydrogenase | Secreted | LDHA | 3939 | 1 | | | | | | | | |
| M30                    | Cytokeratin 18 fragments | Secreted | KRT18 | 3875 | 1 | | | | | | | | |
| Midkine                | Growth factor | Secreted | MDK | 4192 | 1 | | | | | | | | |
| MMP2                   | Matrix Metalloproteinase 2 | Secreted | MMP2 | 4313 | 1 | | | | | | | | |
| MMP8                   | Matrix Metalloproteinase 17 | Secreted | MMP17 | 4316 | 1 | | | | | | | | |
| MMP9                   | Matrix Metalloproteinase 9 | Secreted | MMP9 | 4318 | 2 | 1 | 1 | | | | | | |
| MUC1                   | Cancer Antigen 15-3 | Secreted | MUC1 | 4582 | 21 | 1 | 4 | 13 | 2 | 1 | | | |
| MUC16                  | Mucin 16 or Cancer antigen 125 or 126 | Secreted | MUC16 | 94025 | 3 | | | | | | | | |
| Nectin-4               | Nectin Cell Adhesion molecule 4 | Secreted | NECTIN4 | 81607 | 1 | | | | | | | | |
| NSE                    | Neuron specific enolase | Secreted | ENO2 | 2026 | 1 | | | | | | | | |
| Biomarker Abbreviation | Biomarker | Type of Biomarker | Gene | Gene ID | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|------------------------|-----------|-------------------|------|---------|--------------------------|---------------|-----------------|----------------------|--------------------------|---------------------------|-----------------------|-----------------|----------------------|
| OC                     | Osteocalcin (bone gamma-carboxylglutamate) protein | Secreted | BGLAP | 632 | 3 | 2 | 1 |
| OPG                    | Osteoprotegerin | Secreted | TNFRSF11B | 4982 | 1 | 1 |
| PAI-1                  | Plasminogen activator inhibitor 1 | Secreted | SERPINE2 | 5054 | 1 | 1 |
| PIK3CA                 | Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α | Secreted | PIK3CA | 5290 | 1 | 1 |
| PLG                    | Plasminogen | Secreted | PLG | 5340 | 1 | 1 |
| Prothrombin            | Coagulation factor II | Secreted | F2 | 2147 | 1 | 1 |
| PTH                    | Parathyroid hormone | Secreted | PTH | 5741 | 1 | 1 |
| RANKL                  | Receptor activator of nuclear factor kappa B ligated | Secreted | TNFSF11 | 8600 | 1 | 1 |
| Survivin               | baculoviral IAP repeat containing 5 | Secreted | BIRC5 | 332 | 1 | 1 |
| TATI                   | Tumor associated trypsin inhibitor | Secreted | SPINK | 6690 | 1 | 1 |
| TGF-B1                 | Transcription Growth Factor Beta 1 | Secreted | TGFB1 | 7040 | 4 | 1 | 1 | 2 |
| THBS-1                 | Thrombospondin (TSP-1) | Secreted | THBS1 | 7076 | 5 | 3 |
| TIMP1                  | Tissue inhibitor of metalloproteinase 1 | Secreted | TIMP1 | 7076 | 3 | 2 | 1 |
| TK1                    | Thymidine kinase 1 | Secreted | TK1 | 7083 | 2 | 1 | 1 |
| TNC                    | Tenascin-C | Secreted | TNC | 3371 | 1 | 1 |
| TPA                    | Tissue polypeptide antigen Plasminogen activator, tissue type | Secreted | PLAT | 5327 | 1 | 1 |
| TRACP5s                | Tartrate resistant acid phosphatase 5a | Secreted | ACP5 | 54 | 2 | 1 | 1 |
| TSH                    | Thyroid stimulating hormone | Secreted | TSHB | 7252 | 1 | 1 |
| TWEAK                  | Tumor necrosis factor related weak inducer of apoptosis | Secreted | TNFSF12 | 8742 | 1 | 1 |
| u-PAR                  | Urokinase-type plasminogen activator | Secreted | PLAU | 5328 | 1 | 1 |
| VEGF                   | Vascular Endothelial Growth Factor | Secreted | VEGFA | 7422 | 11 | |
| VEGF-A                 | Vascular Endothelial Growth Factor A | Secreted | VEGFA | 7422 | 5 | 7 |
| VEGF-C                 | Vascular Endothelial Growth Factor C | Secreted | VEGFC | 7424 | 2 | 1 |
| YKL-40                 | Chitinase-3-like protein 1 | Secreted | CHI3L1 | 1116 | 2 | |
| ICTP                   | C-terminal telopeptide of collagen type I | Secreted | CTP2A1 | 1593 | 1 | |
| Fibronectin            | Fibronectin | Secreted | FGA | 2243 | 1 | 1 |
| OFN                    | Osteopontin (Secreted phosphoprotein 1) | Secreted | SPP1 | 6696 | 2 | 1 | 1 |
| Osteonectin            | Osteonectin | Secreted | SPARC | 6678 | 1 | 1 |
| Peristin               | Peristin | Secreted | POSTN | 10831 | 1 | 1 |
| Phosphocholine lipoprotein | Secreted | PCYT1A | 5130 | 1 | 1 |
| VCAM1                  | Vascular endothelial adhesion molecule | Secreted | VCAM1 | 7412 | 1 | 1 |
Table A6. Translational stage of genes investigated in studies that focus on circulating DNA.

| Biomarker Abbreviation | Biomarker | Gene | Gene ID | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|------------------------|-----------|------|---------|--------------------------|----------------|------------------|----------------------|--------------------------|--------------------------|-----------------------|------------------------|---------------------------|
| AKR1B1                 | Aldo-keto reductase family 1 | AKR1B1 | 231     | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| AKT1                   | AKT serine threonine kinase 1 | AKT1   | 207     | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| APC                    | APC, WNT signaling pathway     | APC    | 324     | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| ARHGEF7                | Rho guanine nucleotide exchange factor 7 | ARHGEF7 | 8674    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| COL6A2                 | Collagen type VI α 2 chain | COL6A2 | 1292    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| ESR1                   | Estrogen Receptor 1            | ESR1   | 2599    | 4                        | 4              |                  |                      |                          |                          |                       |                          |                           |
| FGFR1                  | Fibroblast growth factor receptor 1 | FGFR1 | 2260    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| FGFR2                  | Fibroblast growth factor receptor 2 | FGFR2 | 2263    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| GPX7                   | Glutathione peroxidase 7       | GPX7   | 2882    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| GSTP1                  | Glutathione S-transferase 1    | GSTP1  | 2950    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| HER2                   | Human epidermal growth factor receptor 2 | ERBB2 | 2064    | 2                        | 2              |                  |                      |                          |                          |                       |                          |                           |
| HIST1H3C               | Histone cluster 1 H3 family member C | HIST1H3C | 8352    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| HOX84                  | Homeobox B4                   | HOX84  | 3214    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| IDH2                   | Isocitrate dehydrogenase 2     | IDH2   | 3418    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| KU86                   | X-ray repair cross complement 5 | KU86   | 7520    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| PIK3CA                 | Phosphatidylinositol-4,5-bisphosphate 3 kinase catalytic subunit α | PIK3CA | 5290    | 6                        | 6              |                  |                      |                          |                          |                       |                          |                           |
| PTEN                   | Phosphatase and tensin homolog | PTEN   | 5728    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| RARβ2 gene             | Retinoic acid receptor beta    | RARβ   | 5915    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| RASGRF2                | Ras protein specific guanine nucleotide-releasing factor 2 | RASGRF2 | 9924    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| RASSF1A                | Ras association domain family member 1 | RASSF1A | 11186   | 6                        | 6              |                  |                      |                          |                          |                       |                          |                           |
| Statholin              | Statholin                      | SFN    | 2610    | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| TM6SF1                 | Transmembrane 6 superfamily member 1 | TM6SF1 | 53346   | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| TMEFF2                 | Transmembrane protein with FGF like and two follistatin like domains 2 | TMEFF2 | 23671   | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |
| TP53                   | Tumor Protein P53              | TP53   | 7157    | 5                        | 5              |                  |                      |                          |                          |                       |                          |                           |
| WGA                    | Whole genome amplification     | WGA    | *       | 1                        | 1              |                  |                      |                          |                          |                       |                          |                           |

* Whole genome amplification has been performed once. No specific gene or gene ID has been reported as multiple genes can be identified. However, in the study that presented whole genome amplification, no further gene information has been presented.
Table A7. Translational stages of microRNAs.

| Biomarker | Total Number of Articles | Basic Research | Proof of Concept | Comparison of Methods | Basic Predictive Research | Basic Prognostic Research | Prognostic Validation | Observational Trials | Health Economic Analyses |
|-----------|-------------------------|----------------|------------------|-----------------------|--------------------------|--------------------------|-----------------------|------------------------|------------------------|
| miR-10b   | 4                       |                |                  |                       | 3                        | 1                        |                       |                        |                        |
| miR-1260  | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-1280  | 1                       |                |                  |                       | 1                        |                          |                       |                        |                        |
| miR-141   | 2                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-155   | 3                       |                |                  |                       | 3                        | 1                        |                       |                        |                        |
| miR-159   | 2                       |                |                  |                       | 2                        | 2                        |                       |                        |                        |
| miR-17    | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-19a   | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-200a  | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-200b  | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-200c  | 2                       |                |                  |                       | 2                        | 2                        |                       |                        |                        |
| miR-2015  | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-203   | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-21    | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-21    | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-21    | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-21    | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-29b2  | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-34a   | 2                       |                |                  |                       | 2                        | 2                        |                       |                        |                        |
| miR-373   | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-41    | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-720   | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| miR-93    | 1                       |                |                  |                       | 1                        | 1                        |                       |                        |                        |
| Profiling 65 miRs | 1 |                |                  |                       |                          |                          |                       |                        |                        |
| U6/SNORD44 | 1                      |                |                  |                       |                          |                          |                       |                        |                        |
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