Evidence Based Medicine, in Precision Oncology

M. Nezami*, Steve Hager

Pacific Medical Center of Hope, Fresno, USA
Email: *amnezami@yahoo.com

Abstract

The disagreements in clinical data and therapy recommendations extracted from different sources/studies are a common finding in oncology research. Knowingly “biology is less reproducible than physics and mechanic engineering”, in order to overcome the disagreements and to find common grounds, we still rely on meta-analysis and systemic reviews for the highest level of evidence. To gather systemic review data base, a bibliographic search usually is conducted in the PubMed and in Cochrane Central Register of Controlled Trials databases to address a common clinical challenge. That said, frequently due to common conflicts between articles outcomes, an opinion of a third investigator is sought. Here in this article, we propose a rationale that could explain the differences in outcomes as a result of imperfect understanding of the current research database secondary to the unique biology of the tumor, rather than statistical interpretation on findings. We believe that the differences in findings merely are based on blinded inclusion criteria, and lack of accurate companion diagnostics to correlate the magnitude of response to each therapy. The objective of this article is to discuss a strategy to overcome such discordance by providing quantitative biological measures for genomic classification and correlation of tumor response to the selected targeted therapy. We further review such analysis in a case series of Her2 positive breast cancer and conclude that translational research would be clinically relevant when customized to the biological findings.

Keywords

Breast Cancer, Her/Neu 2 Cancer, Targeted Therapies, Precision Oncology, Epigenetic Therapies

1. Background

Classic trial design paradigms are challenged by tumor heterogeneity, as they are
unable to test targeted therapeutics against low frequency genomic “oncogenic driver” aberrations with adequate power. Usual accrual difficulties to clinical trials are exacerbated by low frequencies of any given molecular driver. To address these challenges, there is need for innovative clinical trial designs and strategies implementing novel diagnostic biomarker technologies to account for inter-patient molecular diversity and scarce tissue for analysis [1]. Current attempts in application of new targeted therapies for cancer have been based on the recent understanding of the tumor set of heterogenous colonies which each at certain point of time drives the tumor growth. Determination of these sets of colonies specific genomic alterations by biopsy (tissue and liquid) has provided extensive knowledge in exploring several actionable targets. That said, majority of the current drugs are approved based on the “presence” of a specific alteration rather than its quantitative measure of expression. For example, the presence of EGFR alteration in lung cancer, or Her 2/neu mutation, would prompt administration of EGFR targeted therapies. That said, the activated (mutated) target can “express” itself in a wide range, and more importantly its expression varies by time, and is not static [2] [3] [4]. Such dynamic effect has long been clinically underestimated, as the tumor initial response has been more of a focus than its duration of response. In fact, due to the tumor secondary mutations and changes in its expression of treated target, no one can really know how long a patient will respond to a specific targeted therapy. The amount of response also has only been estimated based on historical data in each study. For tumors that overexpress HER 2, trastuzumab-based chemotherapy is now the standard of care and it has improved the rate of response, time to progression, and the overall survival in patients with metastatic breast cancer, as well as disease-free and overall survival in patients with localized invasive breast cancer. That said, Her 2 positive breast cancer response to Herceptin, (if there is no inherent or acquired resistance reported) has a quantity and duration with a range of negligible to significant and between few weeks to years, depending of the clinical study performed and the candidates specific tumor character included in the studied. Many of the studies that have looked at application of Gleevec for example in Her 2 positive breast cancer with hormonal blockade again disagree on the outcomes as the candidates were not stratified for the presence of TP53 or PDGF. Such discordance and disagreements merely are caused by the lack of biological information on tumor behavior and its unique character in each case studied in the trial. As a result, next generation clinical trials are suggested to replace the current oncology clinical trial design to avoid the blinded conclusions in blinded trials.

Circulatory DNA and Circulatory tumor cells are accessible by a blood sample, and can provide significant information on tumor genomic profile in a dynamic fashion. Such information includes genomic presence and absence of mutations, amplifications and even mRNA findings at CTC level [5] [6]. With such information, it would be very feasible to assess the tumor's signature and
predict the driver cells fingerprint. Again, in the case of Her 2 positive disease, the presence of ERBB2 alterations is reported in the case of both Her 2 positive and Her 2 negative disease (by biopsy), and therefore, can guide the treatment. The quantity of alterations in the circulatory DNA is also available based on the percentage of the alteration found in the c DNA. For example in a Her 2 positive disease, the ERBB2 expression is quantitatively measurable and can guide therapy or change in therapy, in case of Her 2 resistance, long before an imaging study suggests progressive disease [6].

Other relevant example in Her 2 breast cancer, is presence and quantity (percentage) of TP53 c DNA post therapy which can provide information on activation of alternative pathways secondary to using anti Her 2 targeted therapy, and can yield to switching the treatment, or adding m tor inhibitors or PDGF blockers to the therapy.

The Serum Her 2 level is a very informative and prognostic assay. It would provide significant information both on presence and quantity of Her 2 disease, and it also correlates with tumor burden. It is also suggested that serum Her 2 level correlates with Her 2 targeted radio labeled imaging. Such test in addition to the CTC and c DNA can identify the response to anti Her 2 therapy months before a scan is performed, and can meaningfully determine both response and predict resistance to her 2 therapy [7].

It is therefore completely understandable when different trials have completely different results when it comes to the treatment of Her 2 disease, as the therapies are provided to patients whom individualized genomics are unknown. This phenomenon questions the validity of existing literature in clinical oncology when most of the studies and what called as evidence based medicine cannot fully be relied upon, specially for a physician implementing customized targeted therapies for cancer, where whole genome sequencing is applied [8] [9] [10].

2. Method and Material

We prospectively treated 4 cases of stage four Her 2 positive breast cancer patients with targeted therapy and monitored their response. These patients were randomly selected in the population of the clinic. These patients were all white, ages of 37 to 57, average age of 46 years old and all were non smoker. The Her 2 targeted therapy was selected based on clinician’s choice of drug, and profile of expected side effects. The proposed companion diagnostics in this article, (c DNA, CTC, serum Her 2 level) were performed. We showed a wide range of laboratory findings. Presence of circulatory tumor cells and the expression of Her 2 m RNA (through Biofocus laboratory in Germany) were quantified. Circulatory tumor DNA was assessed by Guardant 360 laboratory, and serum Her 2 level were checked through LabCorp, US. We reviewed the response to Her 2 therapy based on the laboratory findings. We were able to successfully improve patients outcome by switching the therapy based on companion diagnostic discussed.
3. Cases

Case 1:

47 years old female with history of stage four breast ductal carcinoma, diagnosed in 2/2014 status post left lumpectomy, refused traditional treatments, including chemo and radiation. Her left breast mass was carrying a low recurrence score on oncotype dx, further she had tried many alternative therapies, including GC MAF with no results, in fact her tumor just progressed and developed extensive bony mets in her scan performed in July 2014. The bony lesions were destructive in all areas involving the thoracic and lumbar spine, too many to count.

She received 7 injections of XGEVA in 2015, and stopped due to the side effects. She was referred by her oncologist to us for evaluation and therapy. Her main complaint was pain and inability to walk. After initial evaluation which consisted of CTC, c DNA and serum Her 2, tumor markers, and LDH, she was immediately started on IV epigenetic therapies (per MTET protocol) which she received on daily basis for two weeks, then tapered to twice a week. Her initial labs on July 12th, showed extensive disease with very high markers (CA 15.3, 27.29 and CEA) over 1200, her LDH 900, her c DNA was positive for PI3K and FGFR1. Her calcium was at 12.4, due to extensive bony mets, showed in her spinal MRI in June 2016.

She reported improved quality of life after the very first treatment, and continued to improve clinically. Her ECOG improved 3 points from 3 to 1. Her labs were repeated and after two weeks on July 26th, and showed a marked reduction (20 percent) in all her tumor markers. Her c DNA completely resolved (please see Figure 1). Her calcium dropped to normal range at 8.3. Her serum Her 2 level dropped down from 124 down to 66, measured on 7/12/16 and 8/5/16 (she had not received any Her 2 drug at this time). Please see Figure 2.

She further developed metastatic dural disease in October 2016, which responded to the therapy with complete resolution of neurological deficits in 12/15/16 after two months of therapy.

Further she was treated with targeted therapies (everolimus) added to Herceptin, as her c DNA showed positive PI3k alteration. Such combination further continued to show improve results. Her tumor markers dropped significantly as her LDH. CA 27.29 dropped down to 804 from 1136 (measured on 8/5 and 8/16/16). Her LDH dropped from 742 to 556. Her Alk-P dropped from 172 to 125. Her CEA dropped from 2368 to 1620, her CA 27.2 dropped from 1136 to 804 and her CA 15.3 dropped down from 954 to 757 (measured on 8/16/16). Her Her 2 continued to drop down this time to 28 (measured on 8/23/16), Her markers are continuing to come down on 1/6/17 with CEA at 635 from 2025. CA 15.3 at 962 from 2172. CA 27.29 at 1114 from 2049, LDH 291 from 556.

Her restaging PET scan shows significant response, to interval therapy including almost complete resolution of her bony mets, decreased sizes and activity on all lesions. Resolution of right humeral head, and lumbar spine, She still has mild persistent left femur SUV activity of 3.0 from 8.4 measured on
Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the relative changes of observed cfDNA at different sample submission time points. The "Somatic Alteration Burden" value below refers to the maximum % cfDNA detected at each time point. Amplifications are not plotted and only the first and last four test dates are plotted. Please see the physician portal for the Tumor Response Map with all test dates.

Somatic Alteration Burden: 1.6% ND

Genomic Alterations: Not Detected (ND). Genomic alterations may be present that are below the limit of detection of this test. Certain sample or variant characteristics may result in reduced analytic sensitivity. Genomic alterations in a tumor may be present, but are not detected in circulating cell-free DNA from this blood specimen with this test.

Summary of Alterations & Associated Treatment Options

The percentage, or allele frequency, of altered cell-free DNA (% cfDNA) circulating in blood is related to the unique tumor biology of this patient. Factors that may affect the amount/percentages of detected genomic alterations in circulating cell-free DNA in blood include tumor growth, tumor size, heterogeneity, vascularization, disease progression, or treatment.

| Alteration | Mutation Trend | % cfDNA | cfDNA Amplification | FDA Approved in Indication | Available for Use in Other Indications | Clinical Drug Trials |
|------------|----------------|---------|---------------------|---------------------------|----------------------------------------|---------------------|
| PIK3CA     | E545K          | 100     | ND                  |                           |                                        |                     |
| FGFR1      | Y701F          | 100     | ND                  |                           |                                        |                     |

For a more detailed Guardant360 Patient Report, log onto: https://portal.guardianthealth.com
To set up an account, contact Client Services: 855.998.8887

The chart above annotates the percentage or allele frequency, of altered circulating cell-free DNA (% cfDNA) detected in this patient. The detected genomic alterations are listed in descending order by % cfDNA by gene.

The "FDA Approved in Indication" and "Available for Use in Other Indications" columns describe drugs associated with specific genomic alterations. It is based on publicly available information as described in the "Detailed Therapy Results" and "Clinical Relevance of Detected Alterations" sections of the report.

Definitions

None.

Figure 1. Circulating DNA (Pre and Post therapy).
Figure 2. Serum Her2 level.
10/30/15, and proximal humerus at 2.7 from 4.3 and residual right breast at 1.2. Left thyroid at 2.5 from 3.8 (please see Image 1).

Case 2:

50 years old female with history of Her 2 positive breast cancer who had not had a biopsy yet, when she referred to us, but her tumor markers were elevated after she found a mass in her left breast growing for over a year. She has done a thermogram which was suspicious. Upon her arrival, we ordered biomarkers as well as a staging PET scan which showed significantly high metabolically active tumor in her left breast as well as axilla, and her iliac bones as well as Thoracic and lumbar spine. (SUV activity of 9.9) Her tumor markers were highly elevated in hundreds and she had positive c DNA on her liquid biopsy which was positive for PI3k as well as c KIT, measured on 11/19/15. Her TGF was also elevated, expected form her c KIT overexpression. Her CTC came back positive for c MYC, and were positive for ERBB2 (Her 2).

She was started on combination of MTET and Herceptin. Her TGF also dropped from 24000 measured on 1/19/16 down to 1258 on 2/22/16. Her cancer was restaged on 4/1/16, through a whole body PET which showed a near complete resolution of thoracic LNs, FDG activity, as well as complete resolution of FDG activity in T3 and L4 bony mets (SUV dropped to 2.0 compared to 9.1). A significant reduction of both extend and the level of metabolic activity was seen at these levels. There were reported pulmonary nodules with remaining activity.

Further she was started on Afinitor in December 2016. Her c DNA was repeated, and it showed stable Kit, and resolution of PI3K to none detectable. (Please see Figure 3). Further c DNA showed reduction of MAF to 0.5. Her restaging PET scan on 1/3/17, showed improvement in both size and activity of all metastatic lesions in the lungs, and bony pelvis. No new lesion was seen. The left upper lobe lung lesion decreased in size from 1.4 to 1.2 cm, and SUV from 12.6 to 4.9, left upper lobe lesion 1 cm from 1.6 cm, activity down from 11.7 to 1.1, right upper lobe lung lesion 0.9 cm from 1.3 cm, activity down from 10 to 3.7, axillary nodes SUV of 0.9. left iliac wing lesion decreased SUV activity from 8.3 to 4.7. left ilium decreased from 9.9 to 4.5. inferior pubic ramus decreased from 5.3 to 3. L4 lesion stable.

She continues the treatments at our clinic with improved clinical response.

Image 1. Pet Scan before and after the therapy.
**Figure 3.** Circulating DNA (before and after the therapy).
Case 3:

37 years old female with history of breast cancer diagnosed in 1/2014, stage IIA, with ER/PR/Her 2 all positive, refused all conventional therapies, including hormonal blockade, opted for alternative treatments in Mexico, have exhausted IPT, Vit C drip and other alternative treatments, including autologous immune cell therapy and hyperthermia, referred to us by her PMD, status post mastectomy in 1/2014, and recurrence of the tumor in 9/2015 at the surgical implant replacement, in Cleveland clinic, with lastly November 2015 scan which confirmed presence of innumerous bony mets as well as thoracic and neck LN involvements, extensive chest and bone disease. Upon her evaluation we found significantly high tumor markers as well as IGF-1 and positive circulatory DNA in her liquid biopsy with a mutated P53 as well as ERBB2. Her Serum Her 2 was also elevated. Her scan on 10/7/15, showed right breast mass with SUV of 14.8, as well as several axillary, mediastinal and hilar lymphadenopathies ranging in SUV of 7 - 9.6. Many sclerotic bony lesions in her glenoid, sternum and sacrum (SUVs ranging between 7.8 to 9.2).

She then was started on Herceptin along with IV epigenetic therapies which she received on daily basis. Her IGF-1 was repeated after two weeks of therapy and it reduced from 240 to 193 (measured on 11/9/15 and 11/23/15, respectfully). Her quality of life improved while she had not changed her diet or supplements.

She started Herceptin, based on both the tumor molecular profiling showing overexpression of Her 2. She concurrently received IV epigenetic therapies, based on again the molecular profiling of her tumor showing overexpression of TET2.

Her CTC was repeated by biofocus on 12/17/15 and it showed complete eradication of CTC with CK20 and ERBB2. The C Myc virtually stayed the same. (Please see below). Her LDH also dropped from 303 to 147 after just three days of treatment (measured on 12/25 and 2/28/15 respectively).

On 1/20/2016, her CTC was repeated and it showed complete resolution of CTC post therapy. (Please see Figure 4). Her circulatory DNA was measured through Guardant and it was positive for PI3k and TP53, at high MAF, measured on 11/9/15, 12/1/15 and negative on 12/30/15 (please see Figure 5). She received Herceptin along with IV multitargeted epigenetic therapies, that could explain the disappearance of ERBB2 from her CTC and/or c DNA, however there is no expected positive effects from Herceptin on P53, or PI3K, nor the c MYC present at her initial CTC. We contribute such finding to the IV epigenetic therapies, she received at the interim, as this therapy in fact targets c MYC, P53 and Pi3k simultaneously.

She stopped the IV epigenetic therapies on 1/16, as she moved back home, and stayed on Herceptin every three weeks, schedule, along with monthly Lupron and Xgeva, and tamoxifen. Her restaging scan in August 2016, showed significant progression of her disease with increased sizes and metabolic activities.
in all her lesions in thoracic and bones (New L 1 lesion with SUV activity of 16.9). Sternum enlarged tumor with SUV of 16.9 from 5.6, Sacrum lesion enlarged with SUV activity of 16.9 from 5.6, Right sacrum from 7.4 down to 3.7, Scapula from 5.2 to 13.1.

At this point the CTC was repeated and it showed the presence of HER 2 positive cells again. She immediately returned to our clinic and started the IV therapies again on 8/25/16, on daily basis. Her restaging scan was ordered on 9/15/16, and showed partial response to therapy with significant reduction in all her lesions sizes and SUV metabolic activities (cervical node, right breast implant 8.2 from 12.2, axillary nodes 4.2 from 6.0, Pulmonary (hilar) nodules 4.3 from 9.2, and bony lesions in sternum 10.2 from 16, L1, from 16.3 down to 11.9. right scapula from 13.9 down to 8.4, sacrum from 7.4 to 6.6).

Her CTC again repeated in December 2016, showed complete resolution of the CTC post therapy (see Figure 6).

This concept is novel as it could also substantiate our theory of targeted epigenetic therapies for driver epimutations in cancer, in this case could well correlate with the mutation identified at TET2 in the molecular profiling of this tumor.

**Case 4:**

57 year old female with breast invasive ductal carcinoma, first diagnosed in October 2000. She was treated with alternative treatments in Mexico and remained in remission until 2008, when she had right breast pain. She went back to Mexico and had right mastectomy. Her pathology showed ER/PR/HER 2 triple positive with a Ki67 of 60%. After that, she received Herceptin and hormonal blockade with arimidex. Her PET scan done at that time confirmed
Figure 5. Circulating DNA.
Analysis Report of Lab No. 4402037443 from 07.12.2016
Patient: Breast CA

For the analysis, we performed the following work steps

1. Isolation of circulating tumor cells / micrometastases

In order to obtain circulating tumor cells from the patient’s peripheral blood, large cells and cell-clusters as well as epithelial cells were isolated. A preparation of mononuclear cells (MNC) served as a control cell fraction. From all fractions mRNA was isolated. Afterwards, the expression of tumor-relevant genes was measured by quantitative real-time RT-PCR.

2. Molecular detection of circulating tumor cells

The following molecular markers were used to detect tumor cells:

- **Telomerase**
  - The expression of the telomerase-gene can be increased in most tumor types, but not in normal tissue. An increased expression of the telomerase gene may be indicative for the presence of tumor cells in the circulation.
  - neg: Expression of telomerase was not detected in the isolated cells.

- **C-MYC**
  - Overexpression of C-MYC indicates an increased proliferation-rate of the isolated cells. An increased proliferation-rate is a typical feature of tumor cells.
  - neg: Expression of C-MYC was not detected.

- **ERBB2**
  - Overexpression of ERBB2 (HER2/Netu) is a trait of different types of cancers and may be observed also in breast cancer. Thus, the detection of ERBB2 overexpression may be indicative for the presence of circulating tumor cells.
  - neg: The expression level of ERBB2 was not elevated.

- **CK19**
  - The detection of an expression of the cytokeratin 19 (CK19) gene indicates the presence of epithelial cells and may thus be indicative for circulating tumor cells.
  - neg: There was no expression of CK19 detected.

Interpretation

In the fraction of isolated tumor cells, abnormal expression of all measured tumor-associated marker-genes was not observed.

Conclusion

According to the panel of molecular tumor markers used for this analysis, there are no indications for presence of cancerous cells in the analyzed blood specimen.

3. Comparison of present findings with former results

Compared to the former analysis from 17-Dec-2015, the expression level of the molecular tumor marker C-MYC has significantly decreased and is in the normal range now, as the other markers too.

| Marker | ERBB2 | C-MYC | Telomerase | CK19 |
|--------|-------|-------|------------|------|
| Threshold | (2.0) | (2.0) | (2.0) | (>1) |
| 20.01.2016 | 1.9 | 0.28 | 0 | 0 |
| 17.12.2015 | 1.91 | 0.37 | 1.83 | 0 |
| 02.12.2015 | 12.64 | 2.45 | 0 | 537 eq |

*markers above threshold in bold face, eq = cell equivalents

Conclusion:

The measured values of the detection markers suggest that the tumor cell burden in blood has decreased compared to the former analysis. There are currently no indications for presence of cancerous cells in the analyzed blood specimen.

**Figure 6.** Circulating tumor cells.
metastatic disease with bone involvement. She then was treated at St. Judes where she continued hormonal blockade, herceptin, zometa. She progressed slowly and was under the care of oncologist in Hanford. She also tried and failed Xeloda. She was started on Tamoxifen and offered radiation. Most recently she had been on Femara, but her most recent PET scan on 6/8/16 again showed progression of disease with complete destruction of the sternum. There were multiple lung mets, as well as extensive disease in the peritoneum. There were no liver lesions noted on her most recent PET. She has never had a paracentesis. She referred to us seeking alternative and integrative treatments. Upon her arrival she was evaluated and her labs drawn, which showed presence of CTC in the blood, along with expression of ERBB2 and c Myc. These CTC were ER negative (see Figure 7).

Her molecular profiling was performed which showed presence of several mutations in her tissue biopsied from her humerus. First they were Her 2 negative, second they were ER positive. Third they were MSH-6 positive making them responsive to the IV epigenetic therapies which started immediately on a daily basis.

Her labs reported decreased tumor markers, CA 27.29 at 685 from 765, CA 15.3 at 533 from 574, Her 2 at 75 from 98, measured on 6/28/16 after two weeks of therapy. Her cDNA showed a marked reduction from 7.4 to 5.0 at the level of PI3k. Further as repeated it showed more reduction from 5 to 1.9, measured on 8/2/16 (please see Figure 8).

She received Herceptin after two weeks of MTET. Her initial response to MTET was reduction of her serum Her 2 level (please see Figure 9).

She did not respond to either Lapatinib or Herceptin, as her serum Her 2 increased again. This is a case of inherent resistance to Herceptin, and therefore she was started on epigenetic therapies again this time, with decreased serum Her 2. It was decided to use Afatinib (a pan EGFR blocker) in conjunction with Herceptin, in her case. Her tumor markers dropped and she continued to respond to therapy with improved markers, and continues to improve.

4. Conclusion

In all cases studied, there was a significant correlation between the mutational allele fraction (and presence) of cDNA, positive Her 2 CTC and serum Her 2 level with the magnitude of response. We believe that further hypothesis can be generated by this small sample looking at companion diagnostics of Her 2 disease before, during and after therapy. It is also mentionable that there was some correlation between the serum Her 2 level and resistance to targeted therapy. We believe that the current standard of care should change in regards to initiation and the duration of Her 2 therapy, as relying on the progression of disease in scan, as an end point for the therapy could postpone sequencing or selecting a more desirable therapy. Furthermore these cases substantiate the evidence on effectiveness of multi targeted epigenetic therapies for driver epimutations in cancer.
Figure 7. Circulating tumor cells.
Figure 8. Circulating DNA.
Figure 9. Serum Her level.
Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Catenacci, D.V.T. (2015) Next-Generation Clinical Trials: Novel Strategies to Address the Challenge of Tumor Molecular Heterogeneity. *Molecular Oncology*, 9, 967-996. [https://doi.org/10.1016/j.molonc.2014.09.011](https://doi.org/10.1016/j.molonc.2014.09.011)

[2] Kuwada, S.K., Li, X.F., Damstrup, L., Dempsey, P.J., Coffey, R.J. and Wiley, H.S. (1999) The Dynamic Expression of the Epidermal Growth Factor Receptor and Epidermal Growth Factor Ligand Family in a Differentiating Intestinal Epithelial Cell Line. *Growth Factors*, 17, 139-153. [https://doi.org/10.3109/08977199909103522](https://doi.org/10.3109/08977199909103522)

[3] Erfani, P., Tome-Garcia, J., Canoll, P., Doetsch, F. and Tsankova, N.M. (2015) EGFR Promoter Exhibits Dynamic Histone Modifications and Binding of ASH2L and P300 in Human Germinal Matrix and Gliomas. *Epigenetics*, 10, 496-507. [https://doi.org/10.1080/15592294.2015.1042645](https://doi.org/10.1080/15592294.2015.1042645)

[4] Myers, M.B. (2016) Targeted Therapies with Companion Diagnostics in the Management of Breast Cancer: Current Perspectives. *Pharmacogenomics and Personalized Medicine*, 9, 7-16. [https://doi.org/10.2147/PGPM.S56055](https://doi.org/10.2147/PGPM.S56055)

[5] Park, H.S., Han, H.J., Lee, S., Kim, G.M., Park, S., Choi, Y.A., Lee, J.D., Kim, G.M., Sohn, J. and Kim, S.I. (2017) Detection of Circulating Tumor Cells in Breast Cancer Patients Using Cytokeratin-19 Real-Time RT-PCR. *Yonsei Medical Journal*, 58, 19-26. [https://doi.org/10.3349/ymj.2017.58.1.19](https://doi.org/10.3349/ymj.2017.58.1.19)

[6] Xu, Y., Yao, L., Li, H., Ouyang, T., Li, J., Wang, T., Fan, Z., Lin, B., Lu, Y., Larsson, O. and Xie, Y. (2006) Presence of erbB2 mRNA in the Plasma of Breast Cancer Patients Is Associated with Circulating Tumor Cells and Negative Estrogen and Progesterone Receptor Status. *Breast Cancer Research and Treatment*, 97, 49-55. [https://doi.org/10.1007/s10549-005-9086-7](https://doi.org/10.1007/s10549-005-9086-7)

[7] Tchou, J., Lam, L., Edwards, C., Ky, B. and Zhang H.T. (2015) Monitoring Serum HER2 Levels in Breast Cancer Patients. *SpringerPlus*, 4, 237. [https://doi.org/10.1186/s40064-015-1015-6](https://doi.org/10.1186/s40064-015-1015-6)

[8] Nakagawa, H. and Fujita, M. (2018) Whole Genome Sequencing Analysis for Cancer Genomics and Precision Medicine. *Cancer Science*, 109, 513-522. [https://doi.org/10.1111/cas.13505](https://doi.org/10.1111/cas.13505)

[9] Catenacci, D.V.T. (2015) Next-Generation Clinical Trials: Novel Strategies to Address the Challenge of Tumor Molecular Heterogeneity. *Molecular Oncology*, 9, 967-996. [https://doi.org/10.1016/j.molonc.2014.09.011](https://doi.org/10.1016/j.molonc.2014.09.011)

[10] Lai, T.L., Lavori, P.W., Shih, M.C. and Sikic, B.I. (2012) Clinical Trial Designs for Testing Biomarker-Based Personalized Therapies. *Clinical Trials*, 9, 141-154. [https://doi.org/10.1177/1740774512437252](https://doi.org/10.1177/1740774512437252)