HHS Public Access

Author manuscript

Int J Gynecol Cancer. Author manuscript; available in PMC 2020 June 26.

Published in final edited form as:

Int J Gynecol Cancer. 2009 December ; 19(Suppl 2): S2–S6. doi:10.1111/IGC.0b013e3181c03929.

Proteomic Profiling in Ovarian Cancer

Geoffrey Kim, MD[*], **Lucas Minig, MD**[†], **Elise C. Kohn, MD**[*]

[*]Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD

[†]Radiation Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD

Abstract

Objective: To describe the role of proteomic profiling in the diagnosis and treatment of ovarian cancer.

Methods: We report a thorough review of the literature, ongoing trials, and our group's experience with proteomic profiling for early detection, recurrence, and treatment of ovarian cancer.

Results/Conclusions: Ovarian cancer remains the deadliest gynecologic malignancy in the western world and is most often diagnosed at a rarely curable late stage. Novel applications of proteomic techniques, such as mass spectrometry, show promise in the quest for reliable multimodality screening programs for the early detection of ovarian cancer. Proteomic analysis of tissue samples has underscored the heterogeneity of this disease process. Development of validated assays that survey the genetic and/or proteomic makeup of an individual tumor will add greatly to the histological classification of the tumor and may lead to different treatment approaches tailored to the unique expression pattern of each individual patient. As novel agents that disrupt signal propagation develop, proteomic profiling by reverse-phase protein arrays can characterize the in-tumor efficacy of the agent by quantification of the changes in expression levels of activated proteins. Together, better understanding of the potential diagnostic and therapeutic targets followed with proof-of-target effect will lead to rational combinations of novel therapy and improve individual ovarian cancer patient outcome.

Keywords

Ovarian cancer; Proteomics; Tissue microarray; Mass spectrometry

Ovarian cancer remains the leading cause of death from gynecologic malignancy among US women, with a lifetime probability of developing the disease of 1 in 59. In 2008, approximately 21,650 women were diagnosed with ovarian cancer, and 70% will have died of their disease.[1] Most women will be diagnosed with advanced-stage disease, and with current treatment modalities, the 5-year survival rate is 30% to 40% with advanced-stage disease versus 90% to 95% with organ-confined disease.[2] Thus, early detection of ovarian cancer itself could have a great effect on improved survival. Unfortunately, no validated or

Address correspondence and reprint requests to Geoffrey Kim, MD, Medical Oncology Branch, Center for Cancer Research, National Cancer, Institute, Bethesda, MD 20892. kimgeof@mail.nih.gov.

cost-efficient screening program exists. The traditionally assessed methods of physical examination, CA125 testing, and transvaginal ultrasound lack the necessary sensitivity and specificity to provide accurate detection of early-stage disease. Preliminary results from an organized application of CA125 and imaging are discussed in the article by Jacobs and Menon[3] elsewhere in this compendium. Thus, ovarian cancer is an ideal candidate for the development of an effective screening program, and application of new technologies is essential to discover other effective diagnostic tools.

Proteomics characterizes the proteins and the associated protein and peptide modifications that make up the complex signaling networks that mediate all processes of cellular activity. It also investigates the role of proteins in defining, describing, predicting, diagnosing, and prognosticating disease. The marriage of investigative proteomics and translational proteomics has been slow and continues to advance toward clinical application, with a strong focus on ovarian cancer. The potential targets of proteomics are broad. They can be focused on identifying novel proteins, clustering expression patterns of known proteins, or quantifying expression levels of proteins and their various posttranslational modifications. Application of proteomics for development and validation of screening biomarkers, prognostic biomarkers and biomarker panels to predict behavior during development, progression, or treatment of cancer, and vetting molecular targets or target pathways is currently underway.

Proteomics can also be applied to investigate proof of principle and to illustrate in-tumor mechanism of therapeutic targets. Metrics are needed to prioritize developments in therapy of advanced and recurrent ovarian and other cancers. Proteomic profiling may be applied to body fluids and tissue samples to investigate successful signal interruption with novel tyrosine kinase inhibitors and monoclonal antibodies (Fig. 1). This “bench-to-bedside-to-bench” approach may speed discovery of key signaling pathways, identify potential therapeutic combinations, and allow validation of target hypothesis of signal transduction inhibitors.

PROTEOMIC PROFILING FOR DIAGNOSIS OF OVARIAN CANCER

Biomarkers for Early Detection of Ovarian Cancer

The capacity of new proteomic technologies to analyze heterogeneous biological samples and detect specific proteins can be remarkably powerful. However, the use of these technologies in screening for ovarian cancer is dependent on the discovery and validation of reliable serum biomarkers. A biomarker is a measurable or assessable entity that provides diagnostic, prognostic, or treatment-orienting information that can drive patient care.[4] The National Cancer Institute’s Early Detection Research Network described a 5-step system of biomarker discovery and evaluation: the preclinical exploration to identify promising candidate biomarkers; a clinical assay to determine the ability of the test to detect the disease; a retrospective/longitudinal determination of a putative biomarker’s ability to detect preclinical disease; a prospective screening to identify the extent and characteristics of disease detected by the test and the false-positive rate quantification of the impact of screening on reducing the burden of disease on the population.[5] Many promising markers have been identified and are currently in the first 2 steps of the Early Detection Research

Network discovery and evaluation system. The community agrees that this approach must be rigorously advanced using independent and blinded sample sets, preferably from a variety of sources, to reduce or minimize collection bias.[6]

Seeking new biomarkers via analysis of the whole proteome is difficult because proteomes differ between cells and can be constantly altered by genomic and environmental interactions.[7] Useful biomarker-based screening tests must have several key characteristics. First, the test must be easy to administer. Ideally, the biomarker should be present in an accessible specimen, such as the body fluids, blood, serum, or urine, yet it must reflect the unique changes ongoing in the organ of interest. Furthermore, the test must be both sensitive and specific. Specificity is critical to minimize false-negative procedures. Ovarian cancer is predominantly a cancer of postmenopausal women, with 1:2500 women diagnosed in her lifetime. A biomarker or screening procedure with a 98% specificity would lead to 49 women undergoing an invasive surgical procedure unnecessarily and 1 case of cancer identified. For this reason, it has been estimated that a specificity of 99.6% with a reasonable sensitivity is required.[3] Sensitivity is inadequate in isolation. For example, it has recently been demonstrated that the Risk of Ovarian Cancer Algorithm and transvaginal ultrasound can improve the positive predictive value of CA125 in ovarian cancer screening. However, a final analysis of ongoing screening is required before drawing conclusions on the effect of screening on mortality.[8] The advocates and health practitioners in the gynecologic cancer community agree that minimizing unnecessary operations (optimizing the positive predictive value) in the face of good sensitivity—selective identification of ovarian cancer over other diseases—is optimal.[5]

Researchers are currently putting efforts into identifying combinations of markers that could improve the sensitivity and specificity for the diagnosis of early-stage ovarian cancer. Studies assessing serum levels by radioimmunoassays have compared 2 or 3 markers at a time, showing an increased sensitivity but with an associated decreased specificity.[9] Other investigators have evaluated a novel cytokine rolling circle amplification microarray to assess this tool for the early detection of ovarian cancer. Rolling circle amplification microarray analysis was used initially to determine the levels of 169 proteins in serum from 28 healthy women, 18 women newly diagnosed with epithelial ovarian cancer, and 40 women with recurrent disease (all stages III–IV). The blinded validation was performed with 106 healthy/100 cancer patients (24 early stages). Results were analyzed by using 3 different classification algorithms and a binary code methodology. No single protein could distinguish the cancer group from the healthy controls, and the combination of 4 analytes, leptin, prolactin, osteopontin, and insulin-like growth factor II showed a specificity of 95%, far from the targeted 99.6%.[10] In addition, validation against true early stage, benign pelvic masses, breast cancer, and other confounding cancers is needed. Hence, to date, no properly powered and validated biomarker(s) or biomarker signatures have been identified.

MS for the Detection of Novel Screening Biomarkers

Traditionally, candidate biomarkers have been identified using crude discovery techniques such as enzyme-linked immunosorbent assays and Western blot analysis of known proteins, 2-dimensional gel electrophoresis and identification of putatively changed peptides, and

gene array analysis. Some are moderately high throughput, and all require cumbersome and slow validation. However, these techniques require knowledge of the target protein or gene before application. Mass spectrometry (MS), however, allows for the detection of novel proteins and peptides by their unique "signature," which may be differentially expressed in the cancerous state as compared with normal and may, therefore, not require sequence identification.

Several groups are using MS to evaluate differential expression patterns of proteins and peptides in patient serum or other biospecimens.[11,12] With MS, samples are ionized and detected by an ion detector plate. The time required for the sample to reach the detector plate is a function of the mass-to-charge ratio (m/z), which is unique to each individual peptide or protein.[13] Matrix-assisted laser desorption and ionization with time-of-flight (TOF) and surface-enhanced laser desorption and ionization with TOF (SELDI-TOF) are the most commonly methods used (Fig. 2). Matrix-assisted laser desorption and ionization with TOF uses a matrix that traps a subset of proteins in the sample, which is subsequently ionized and analyzed by TOF. Conversely, SELDI-TOF uses a commercial chip customized with specific bait molecules that either chemically bind protein samples using cationic or hydrophobic interactions or use an antibody to which samples bind followed by a matrix to facilitate ionization.[14] Analysis of spectral images is performed by a software that is capable of segregating different patterns of proteomic peak intensities, and other approaches include sequence analysis of the MS findings. Quality control is critical when applying proteomic techniques to patient samples, and as with all high-throughput technologies that generate large data sets, this approach requires complex and varied bioinformatics methods.[15] The implementation of at least 3 different statistically based algorithms is suggested to obtain reproducible and robust findings, thus, reducing the problem of overfit bias that has been observed in many data-rich analyses.[6]

A large Gynecologic Oncology Group trial, GOG-220, was designed to generate and then validate a proteomic profile (signature pattern) that can diagnose ovarian carcinoma on the background of a pelvic mass. Serum samples have been collected from more than 2000 women undergoing surgical intervention for an undiagnosed pelvic mass. The objectives are to create protein pattern signatures that differentiate malignant ovarian disease from benign or nonovarian malignancy in presurgical specimens using MS and complex analytical bioinformatics. This trial was designed to yield independent discovery and validation cohorts and was prospectively powered to give a 96% specificity and sensitivity in the validation stage. Laboratory work is primed to start. Secondary objectives include differential analysis of early- versus late-stage disease and histotypes, postoperative residual disease, and prognostic outcome.

OVARIAN CARCINOMA SUBTYPES ARE DIFFERENT DISEASES: IMPLICATIONS FOR BIOMARKER STUDIES

Ovarian cancer is an extremely heterogeneous disease creating a large challenge to biomarker discovery. Recently, the World Health Organization has introduced the new classification of ovarian carcinomas divides into 5 types: high-grade serous, low-grade

serous, endometrioid, mucinous, and clear-cell carcinoma.[16] Protein and gene analyses are ongoing to create more consistent diagnostic criteria with which to support this new classification. Köbel et al have retrospectively studied 21 candidate tissue biomarkers in a cohort of 500 women without residual tumor after primary cytoreduction.[17] The authors evaluated these 21 prospective biomarkers with tissue microarray immunohistochemical analysis. Expression analysis revealed that 10 of the 21 proteins were differentially expressed in-between stages. In comparison, 20 of the 21 proteins studied were differentially expressed across subtypes, suggesting that distinct biochemical events are more associated with subtype as opposed to stage. Differences have also been reported at molecular and genetic level among women with the same histological type and stage of ovarian cancer and is presented by Birrer and colleagues in another article in this series.[18,19] This fact may explain why these patients have different responses to chemotherapy and different survival outcomes. Further studies are needed to validate these findings and to determine whether these genetic and proteomic differences require different treatment approaches.

PROTEOMIC PROFILING FOR TREATMENT OF OVARIAN CANCER

Despite these recent advances in biomarker development for the early detection of ovarian cancer, most cases are still diagnosed at a late stage. Novel small-molecule tyrosine kinase inhibitors and monoclonal antibodies have now been developed that specifically interrupt cellular signals driving cancer cell survival, growth, proliferation, invasion, and metastasis. Proteomic analysis of clinical samples obtained during well-designed clinical trials using these novel agents addresses the complexity of the signaling events associated with advanced ovarian cancer. Several fundamental questions need to be addressed when rationally designing therapy with signal transduction inhibitors:

1. Is the target present?
2. Is the target affected by the intervention?
3. Is the target effect sufficient to yield a change in clinical outcome?

Traditional analysis of target modification has been carried out with semiquantitative immunoblotting, immunohistochemical staining, in situ hybridization, enzyme-linked immunosorbent assays, or with other nonstandardized methods. These methods are labor intensive, time consuming, and are often reliant on subjective analysis. However, recent technologies using protein microarrays have allowed quantification of multiple end points including expression levels of key proteins and their activated forms that compose critical signaling nodes involved in proliferation, survival, and angiogenesis.[20,21]

Protein microarrays can be divided into 2 different formats: forward-phase arrays and reverse-phase protein arrays (RPPA).[22] A capture molecule, usually an antibody, is immobilized on a substratum, and the sample of interest is incubated with the array with each spot conferring to a different bait protein in the forward-phase array. The RPPA format applies the unknown biosample to the substratum, and the array is incubated with antibodies or other detectors to defined protein(s). This technique has undergone extensive optimization of sample handling, protein extraction, sample printing, antibody validation, and signal quantification.[22,23]

We applied the RPPA to evaluate biochemical signaling events of the targeted agents imatinib and gefitinib in recurrent and advanced ovarian cancer patients in separate phase II trials.[24,25] In each trial, tumor biopsy was performed before treatment and again after 4 weeks of therapy and used to assess biochemical changes within the tumor. Neither agent demonstrated sufficient clinical activity to warrant further trials or to allow assessment of the relationship between target modulation and outcome. However, the RPPA results demonstrated the presence of the target and the ability of the agent to inhibit the target in the tumor. We concluded that modulation of these targets was insufficient to drive tumor injury with single-agent therapy.

The findings of those studies led to the design and development of a phase I and subsequent phase II study assessing the combination of bevacizumab, a monoclonal antibody against vascular endothelial growth factor, and sorafenib, a receptor tyrosine kinase inhibitor of raf kinase and vascular endothelial growth factor receptor 2. The trial hypothesis is that coupling inhibition of downstream signaling with upstream events would lead to effective disruption of survival pathways in both the cancer cell and cells in the tumor microenvironment. The phase I trial was enriched for patients with recurrent ovarian cancer and demonstrated promising clinical activity in that 6 (43%) of 13 patients had a partial response to treatment.[26] As with the prior clinical trials, the patients underwent biopsy before and after treatment (Fig. 3), and reverse-phase tissue lysate array analysis of the biochemical response in the tumor is ongoing. We eagerly await the analysis of both phase I and phase II trials that will assess whether there are patterns of expression of key proteins that confer sensitivity or resistance to this combination of agents.

FUTURE DIRECTIONS

Proteomic profiling for ovarian cancer shows tremendous promise in addressing the goals of early diagnosis and more effective treatment. The discovery of novel validated biomarker signatures will broaden our understanding of the disease and will further define the driving forces behind this heterogeneous condition. As we gain the ability to delineate the pathways specific to each tumor subset, the targets will be easier to see and thus easier to hit. Ultimately, with the continued collaboration between basic, translational, and clinical scientists, we will reduce the frequency of late-stage disease, improve targeted therapy on an individual basis, and provide better and longer lives for women with ovarian cancer.

REFERENCES

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71–96. [PubMed: 18287387]
2. Ozols RF, Thomas G. Principles and Practice of Gynecologic Oncology. 4th Ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2005.
3. Jacobs IJ, Menon U. Progress and challenges in screening for early detection of ovarian cancer. Mol Cell Proteomics. 2004;3:355–366. [PubMed: 14764655]
4. Azad NS, Rasool N, Annunziata CM, et al. Proteomics in clinical trials and practice: present uses and future promise. Mol Cell Proteomics. 2006;5:1819–1829. [PubMed: 16737951]
5. Mills GB, Bast RC Jr, Srivastava S. Future for ovarian cancer screening: novel markers from emerging technologies of transcriptional profiling and proteomics. J Natl Cancer Inst 2001;93:1437–1439. [PubMed: 11584052]

6. Ransohoff DF. Lessons from controversy: ovarian cancer screening and serum proteomics. J Natl Cancer Inst 2005;97:315–319. [PubMed: 15713968]
7. Stevens EV, Liotta LA, Kohn EC. Proteomic analysis for early detection of ovarian cancer: a realistic approach? Int J Gynecol Cancer. 2003; 13:133–139. [PubMed: 14656269]
8. Menon U, Gentry-Maharaj A, Hallett R, et al. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). Lancet Oncol 2009;10:327–340. [PubMed: 19282241]
9. Bast RC Jr, Urban N, Shridhar V, et al. Early detection of ovarian cancer: promise and reality. Cancer Treat Res 2002;107:61–97. [PubMed: 11775462]
10. Mor G, Visintin I, Lai Y, et al. Serum protein markers for early detection of ovarian cancer. Proc Natl Acad Sci U S A 2005;102:7677–7682. [PubMed: 15890779]
11. Denkert C, Budczies J, Kind T, et al. Mass spectrometry-based metabolic profiling reveals different metabolite patterns in invasive ovarian carcinomas and ovarian borderline tumors. Cancer Res 2006;66:10795–10804. [PubMed: 17108116]
12. Lemaire R, Menguellet SA, Stauber J, et al. Specific MALDI imaging and profiling for biomarker hunting and validation: fragment of the 11S proteasome activator complex, Reg alpha fragment, is a new potential ovary cancer biomarker. J Proteome Res 2007;6:4127–4134. [PubMed: 17939699]
13. Koomen J, Hawke D, Kobayashi R. Developing an understanding of proteomics: an introduction to biological mass spectrometry. Cancer Invest 2005;23:47–59. [PubMed: 15779868]
14. Simpkins F, Czechowicz JA, Liotta L, et al. SELDI-TOF mass spectrometry for cancer biomarker discovery and serum proteomic diagnostics. Pharmacogenomics. 2005;6:647–653. [PubMed: 16143004]
15. Annunziata CM, Azad N, Dhamoon AS, et al. Ovarian cancer in the proteomics era. Int J Gynecol Cancer. 2008;18:1–6.
16. Tavassoeli FA, Devilee P, et al. International Agency for Research on Cancer Pathology and Genetics: Tumours of the Breast and Female Genital Organs. IARC Press, Lyon: World Health Organization; 2003.
17. Köbel M, Kalloger SE, Boyd N, et al. Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. PLoS Med 2008;5:e232. [PubMed: 19053170]
18. Schwartz DR, Kardia SL, Shedden KA, et al. Gene expression in ovarian cancer reflects both morphology and biological behavior, distinguishing clear cell from other poor-prognosis ovarian carcinomas. Cancer Res 2002;62:4722–4729. [PubMed: 12183431]
19. Zorn KK, Bonome T, Gangi L, et al. Gene expression profiles of serous, endometrioid, and clear cell subtypes of ovarian and endometrial cancer. Clin Cancer Res 2005;11:6422–6430. [PubMed: 16166416]
20. Paweletz CP, Charboneau L, Bichsel VE, et al. Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. Oncogene. 2001; 20:1981–1989. [PubMed: 11360182]
21. Davidson B, Espina V, Steinberg SM, et al. Proteomic analysis of malignant ovarian cancer effusions as a tool for biologic and prognostic profiling. Clin Cancer Res 2006;12:791–799. [PubMed: 16467090]
22. Sheehan KM, Calvert VS, Kay EW, et al. Use of reverse phase protein microarrays and reference standard development for molecular network analysis of metastatic ovarian carcinoma. Mol Cell Proteomics. 2005; 4:346–355. [PubMed: 15671044]
23. Winters M, Dabir B, Yu M, et al. Constitution and quantity of lysis buffer alters outcome of reverse phase protein microarrays. Proteomics. 2007;7:4066–4068. [PubMed: 17952872]
24. Posadas EM, Kwitkowski V, Kotz HL, et al. A prospective analysis of imatinib-induced c-KIT modulation in ovarian cancer: a phase II clinical study with proteomic profiling. Cancer. 2007;110:309–317. [PubMed: 17559139]
25. Posadas EM, Liel MS, Kwitkowski V, et al. A phase II and pharmacodynamic study of gefitinib in patients with refractory or recurrent epithelial ovarian cancer. Cancer. 2007;109:1323–1330. [PubMed: 17330838]

26. Azad NS, Posadas EM, Kwitkowski VE, et al. Combination targeted therapy with sorafenib and bevacizumab results in enhanced toxicity and antitumor activity. J Clin Oncol 2008;26:3709–3714. [PubMed: 18669456]

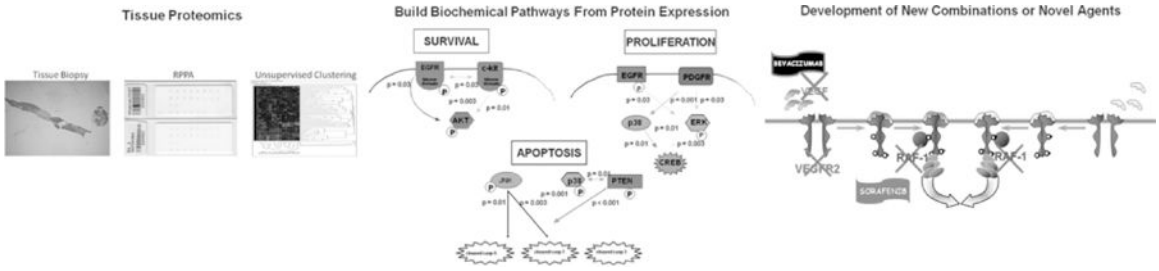

FIGURE 1.

Tissue proteomics for the discovery of novel combinations of signal transduction inhibitors. Proteomic analysis of tissue: tumor biopsies, patient serum, effusion samples, and so on reveals key biochemical pathways involved in cell survival. Rational combination of signal transduction inhibitors targets these pathways for more efficient tumor inhibition.

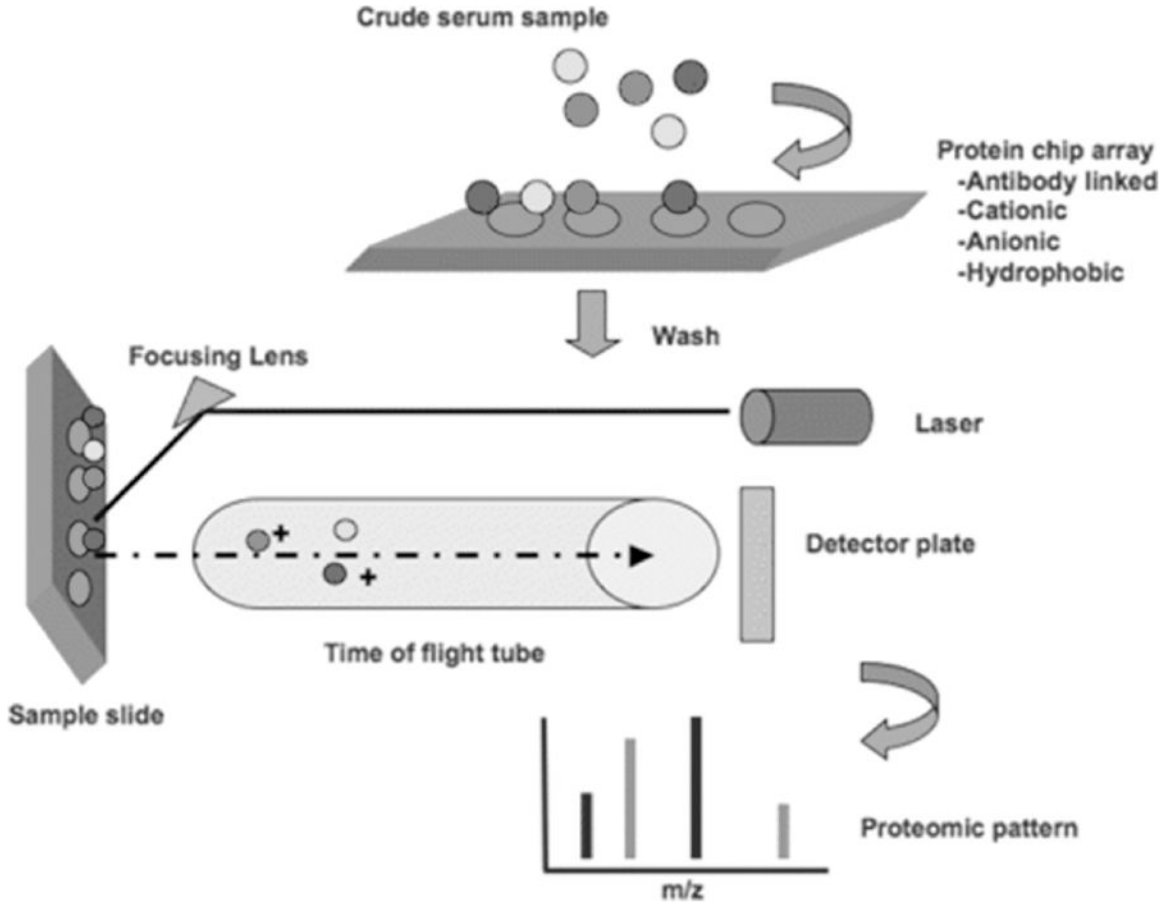

FIGURE 2.

SELDI-TOF. A commercial chip customized with specific bait molecules chemically binds protein samples or uses an antibody to which samples bind. A laser is focused on the captured protein, converting it to the gaseous phase that passes through a tube to a detector plate, where the mass-to-charge ratio is determined.

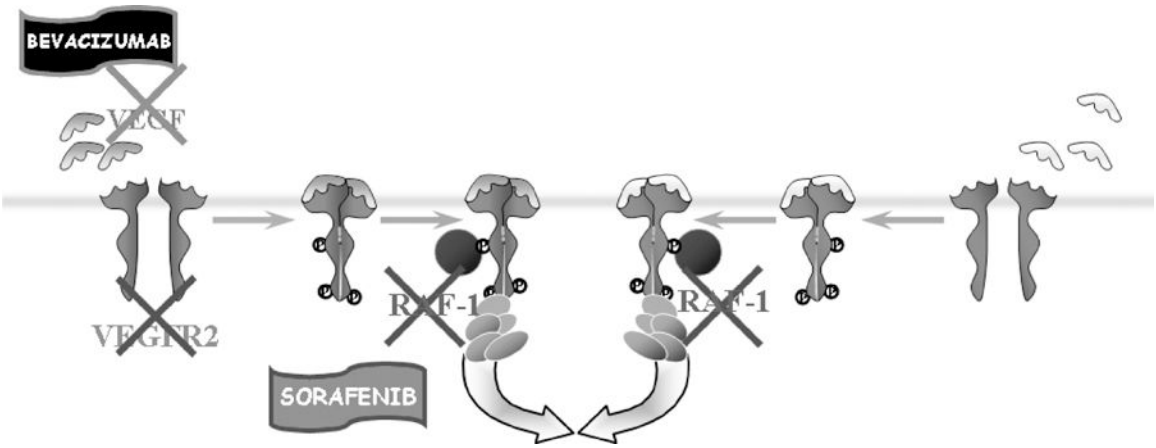

FIGURE 3.

Translational proteomic analysis during treatment on a clinical trial. Serial biopsies are taken before treatment, after treatment with a single agent, and after treatment with both agents to determine biochemical signaling changes in the tumor during treatment. After the computed tomography—guided biopsy is performed, the sample is immediately frozen. Subsequently, the sample is subjected to reverse-phase tissue lysate array.