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Review Article

Circulating Biomarkers for Therapeutic Monitoring of Anti-cancer Agents

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Abstract

Circulating biomarkers have emerged as valuable surrogates for evaluating disease states in solid malignancies. Their relative ease of access and rapid turnover has bolstered clinical applications in monitoring treatment efficacy and cancer progression. In this review, the roles of various circulating biomarkers in monitoring treatment response are described. Non-specific markers of disease burden, tumor markers (eg CA 125, CEA, PSA, etc.), circulating tumor cells, nucleic acids, exosomes, and metabolomic arrays are highlighted. Specifically, the discovery of each of these markers is reviewed, with examples illustrating their use in influencing treatment decisions, and barriers to their application noted where these exist. Finally, opportunities for future work using these circulating biomarkers are discussed.

Key words: biomarker; tumor marker; ctDNA; exosomes; metabolomics; treatment response.

Implications for Practice

In oncology, blood-borne biomarkers are becoming increasingly used for assessing treatment efficacy. A multitude of such surrogates have been studied over the last several decades, each with particular advantages, operating characteristics, and challenges. Practicing oncologists should be aware of existing circulating biomarkers and their recommended use, as well as new technologies on the horizon which may eventually enter routine practice.

Introduction

In the clinical practice of oncology, prompt determination of treatment efficacy is a laudable goal. The swift identification of disease progression on a particular therapy affords patients and their treating clinicians an opportunity to advance onto alternative agents which might offer improved efficacy and limits needless toxicities from non-efficacious treatments. Treatment response of solid malignancies has historically been defined by the absence of clinical symptoms as well as stability/regression of lesions on radiological imaging over time. Given that these are often delayed markers of treatment response, there is tremendous interest in the use of surrogate markers for the early detection of disease progression.

In contrast to prognostic markers, which provide insights into an expected disease course, and predictive markers, which supply information about anticipated response to therapy, biomarkers for therapeutic monitoring offer information as to the efficacy of an *ongoing* treatment. Dynamic changes in the biomarker through therapy provide evidence that a currently applied therapy is having the intended effect of reducing tumor burden or slowing cancer growth. Beyond assessing for disease progression, an ideal therapeutic biomarker also has the potential to be used for determining the optimal duration and dosage of therapy, or for gaining insights into the evolution of tumor biology.

Circulating biomarkers represent particularly attractive surrogates for these purposes and provide numerous advantages in practice (Fig. 1). This review will provide an overview of circulating biomarkers for therapeutic monitoring of anti-cancer agents. In particular, the use of non-specific markers of disease burden, tumor markers, circulating tumor cells, nucleic acids, exosomes, and metabolomic arrays as surrogate markers for treatment response is explored. Finally, challenges in the application of these markers and opportunities for future work are described.

Non-Specific Markers of Disease Burden

Many of the earliest discovered circulating tumor biomarkers were non-specific indicators of disease burden. These surrogates largely consist of naturally occurring macromolecules that are aberrantly upregulated by rapidly dividing cancer cells, or that are released into circulation by loss of cell membrane integrity.[1,2]

Lactate Dehydrogenase

Lactate dehydrogenase (LDH) is one of the most commonly expressed enzymes in nature and plays a physiologic role in

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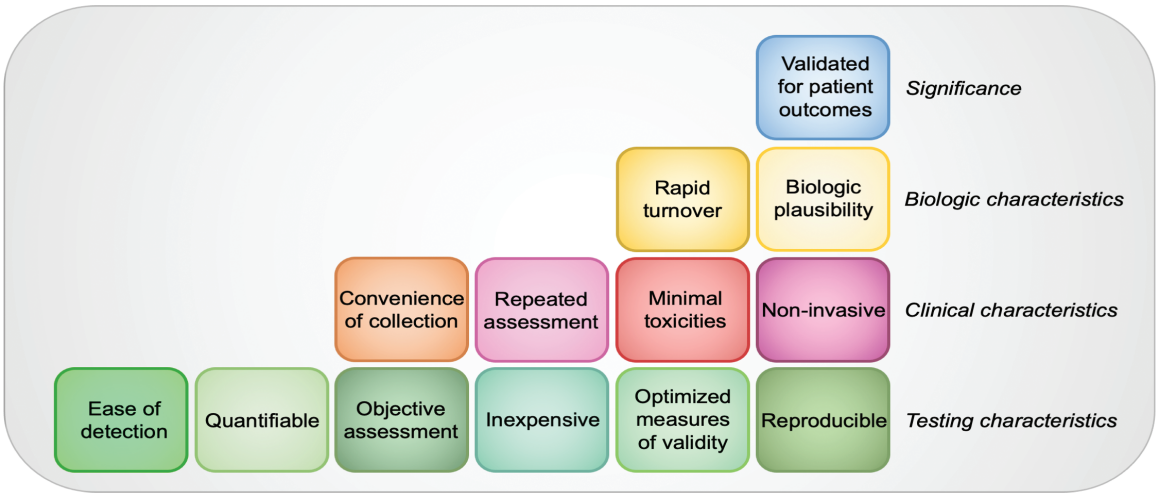

Figure 1. Features of ideal circulating biomarker. In evaluating a circulating biomarker, various factors must be considered. The biomarker must be easily detected, quantifiable, and objectively measured. Detection of the biomarker should be inexpensive. Assays for measurement should have robust validation, with optimized sensitivity and specificity. Further, testing should be reliable/reproducible allowing for results to be standardized across different testing centers. In terms of clinical characteristics, an ideal circulating biomarker is conveniently collected through a procedure which subjects patients to minimal toxicities or adverse effects. The biomarker may be non-invasively detected. There should be opportunities for repeated assessment through a treatment course. For biologic characteristics, biologic plausibility is preferred and may facilitate interpretation and communication around testing results with patients. Rapid turnover is essential for securing real-time responses to ongoing interventions. Finally, the circulating biomarker must be validated in clinical practice and should have proven clinical utility.

anerobic metabolism.[3] The rather ubiquitous expression of LDH across tissue types has led to broad clinical applications in detecting cellular damage across pathologies which include myopathies, myocardial infarction, hemolytic anemias, hepatocellular damage, and cancer.

LDH has been known to correlate with cancer disease burden in pre-clinical and clinical studies for decades, although recent nuances have been described.[4-6] Indeed, LDH is a frequently used stratification factor in clinical trials and has been integrated into prognostic scoring systems (eg, Royal Marsden Hospital score, Gustave Roussy immune score).[7,8] Notably, the predictive value of LDH in melanoma is so robust that it has been incorporated into TNM staging to differentiate M1a/b/c/d (0) from M1a/b/c/d (1) disease.[9]

In terms of dynamic monitoring, LDH is a fairly useful indicator for disease relapse in lymphoma and is included in guidelines from the European Society for Medical Oncology for post-treatment monitoring.[10-12] LDH changes through therapy have been associated with survival outcomes in advanced breast, non–small cell lung, hepatocellular, and testicular cancers.[13-16] In some cases, changes of LDH through therapy have proven superior to baseline LDH values in predicting treatment responses.[16] Although compelling, these observations have been challenged. In certain cohorts, LDH levels through treatment have been found to *not* be correlated with response.[17,18] Others have argued that LDH does not add much to clinical decision making, as patients with increased LDH prior to relapse or progression often have overt clinical symptoms that would have prompted further investigations regardless.[19]

Cell Death Products

Cell death products released into circulation by apoptotic or necrosing malignant cells represent another non-specific marker of disease state. Caspase-cleaved cytokeratin 18 (CK18 M30 and M65) has been studied as a potential surrogate for treatment efficacy in patients with breast cancer during neoadjuvant chemotherapy as well as in advanced gastric cancers among others.[20,21] Other markers under study include HMGB1, RAGE, and DNase.[22] Overall, these molecules provide an indiscriminate measure of cell death in vivo which might reflect on-target killing of neoplastic cells but also includes off-target effects on healthy tissues. At present, these markers are not used in clinical practice.

Neutrophil-to-Lymphocyte Ratio and Other Hematologic Markers

Further non-specific circulating biomarkers aim to explore how immune mediators are associated with responses to therapy. Certainly, the role of the immune system in providing a hospitable environment for cancer growth and key mechanisms underlying this interaction including the "immune checkpoint" (programmed death-1 (PD-1) binding to its ligand PD-L1) have gained recent prominence.[23] Additional factors affecting this interaction, such as the overall extent of somatic mutations present in cancer cells (ie, tumor mutational burden, TMB), have also been described.[24]

Gross metrics for evaluating the immune milieu are routinely collected through cytotoxic therapy follow-up and include ratios of various hematologic cell types (neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio, and monocyte-to-lymphocyte ratio).[25] High NLR predicts worse outcomes for patients with metastatic prostate cancer, localized or metastatic head and neck cancer, and advanced upper-tract urothelial carcinomas.[26-28] Conversely, a low blood NLR is associated with longer survival in patients with metastatic melanoma with brain involvement or advanced non–small-cell lung cancer (NSCLC) treated with PD-1-targeted therapies.[29,30] NLR has also been investigated in aggregate with other markers. Ren *et al* showed among advanced NSCLC patients with TMB > 10 mutations/Mb, those with NLR ≤ 2.5 had significantly more favorable overall survival (OS)

and progression-free survival (PFS) compared with patients with NLR > 2.5.[31] Saravia *et al* have demonstrated that low intratumoural PD-L1/high NLR predicts of lack of response to PD-1 inhibition in patients with advanced lung cancer.[32] Finally, in patients with advanced melanoma receiving ipilimumab, Ferrucci *et al* showed that patients with raised baseline neutrophils and NLR had increased risk of disease progression and death.[33]

Soluble PD-1

Soluble levels of PD-1 (sPD-1) and their dynamics through treatment can reflect anti-cancer immune responses and treatment efficacy.[34] Increased sPD-1 levels have been associated with prolonged survival in patients with nasopharyngeal carcinoma after curative-intent radiotherapy, as well as improved PFS and OS in advanced NSCLC patients receiving anti-PD-1 antibody nivolumab or tyrosine kinase inhibitors against epidermal growth factor receptor (EGFR).[35-37] However, integration of sPD-1 into therapeutic monitoring has been limited by a lack of standardized methods for quantification, and an underdeveloped literature supporting its use in treatment monitoring.

Clinical Considerations

Overall, non-specific markers of disease state are attractive circulating biomarkers for monitoring therapeutic responses. They are often inexpensively collected, frequently have established normal values, and robust methods exist for their detection. These advantages are somewhat mitigated by the non-specific nature of these biomarkers leading to difficulties in their interpretation and an overall lack of prospective, randomized data demonstrating clinical utility.

Tumor Markers

Extensive efforts have been invested into identifying tumor markers with greater specificity in evaluating tumor burden and response to therapy. Biomarkers with variable clinical utility have been proposed, including those that attempt to recapitulate the tumor burden of cancers with certain histopathological origins.

Cancer Antigen 125

Bast et al first suggested CA 125 (MUC16) as a diagnostic marker of epithelial ovarian cancer in 1983.[38] At present, CA 125 maintains a role in monitoring disease response and is recommended alongside imaging criteria for response assessment in relapsed ovarian cancer by the Gynecological Cancer Intergroup.[39] Despite this, the use of CA 125 elevation as a threshold for initiating treatment before radiological disease recurrence has not been linked to an improvement in OS.[40-43] Moreover, the sensitivity of CA 125 declines in heavily treated ovarian cancer that is resistant to platinum chemotherapy, reflecting a potential concerning dynamic change in test performance as cancer biology evolves.[44]

Prostate-Specific Antigen

Prostate-specific antigen (PSA) is a serine protease which is exclusively produced by prostatic epithelium and escapes into circulation in the setting of malignancy.[45] Blood PSA levels are highly specific for disease recurrence and are also directly linked with survival outcomes in advanced disease.[46-49] Serum PSA is routinely used to assess disease response in patients with advanced prostate cancer treated with either chemotherapy or androgen receptor axis-targeted treatment.[47,50] Indeed, PSA is included in standard response assessment criteria in castrate-resistant prostate cancer put forth by the Prostate Cancer Clinical Trials Working Group 3.[51] A notable exception to this would be prostate tumors with neuroendocrine features without significant PSA production.[52]

Carcinoembryonic Antigen

Carcinoembryonic antigen (CEA) is an adhesion molecule involved in signal transduction within adenocarcinomas.[53,54] Increased serum CEA levels have been described in breast, colorectal, gastric, lung, pancreatic, and ovarian malignancies but can also be observed in benign conditions including inflammatory bowel disease, cigarette smoking, diverticulitis, pancreatitis, liver disease, and alcohol consumption.[55-57] Although CEA has a weak role as a screening tool for colorectal cancer (CRC), it has been validated as a prognostic biomarker and surrogate of disease burden pre-operatively for patients undergoing CRC resection, as well as a predictor of disease recurrence during follow-up.[56,58,59] Stable CEA levels are associated with significantly improved PFS in patients with unresectable metastatic CRC.[60-63]

Carbohydrate Antigen 19-9

Although carbohydrate antigen 19-9 (CA 19-9) has also been investigated in CRC, pancreatic adenocarcinoma is perhaps the tumor type in which CA 19-9 has been most extensively studied.[64-67] Decreasing CA 19-9 levels through treatment and persistence of sustainably low levels have both been used in assessing treatment response in both resectable and metastatic disease states.[68-70] However, notable confounding factors have been described, including its relatedness to Lewis blood group antigens and elevation in the setting of obstructive jaundice.[66,71]

Clinical Considerations

Overall, circulating tumor markers are a diverse set of molecules that provide insights into underlying cancer biology. In some cases, these markers have already been established into clinical practice. Presently, CA 125 and PSA are robust predictors in aiding decisions on clinical intervention, as evident by their incorporation into globally accepted disease response criteria for both early and advanced stages of ovarian and prostate cancer, respectively. In contrast, CEA and CA 19-9 have inferior sensitivity and specificity and although they play a significant prognostic and monitoring role in CRC and pancreatic cancer, their impact in clinical decisions is not as strong. Hence, with variable sensitivity and specificity for assessing therapeutic response, only certain tumor markers offer improved performance over non-specific markers of disease burden and are recommended for routine use.

Circulating Tumor Cells

Since their discovery in 1869, our understanding of circulating tumor cells (CTCs) has evolved from a rare complication of solid cancers ("carcinocythemia") to a relatively frequent observation in solid malignancies.[72,73] In the recent literature, CTCs have been evaluated as a correlate in clinical trials where, intuitively, their persistence through therapy uniformly confers an unfavourable prognosis. For example, a recent analysis of 5 prospective randomized clinical trials

of patients with metastatic prostate cancer showed that a decline in CTCs number to zero after 13 weeks of treatment had a higher discriminatory power for OS than PSA.[74] CTCs have also been integrated into composite surrogates alongside other biomarkers (eg, LDH).[75] Subsequent work in metastatic prostate cancer has supported the integration of CTCs into standard measures of assessment when determining prognosis and response to treatment.[76]

Others have explored whether subtypes of CTCs expressing certain proteins might be used in monitoring responses to therapy. Accordingly, CTCs expressing IGF-1R have been monitored in patients with advanced prostate cancer receiving IGF-1R-directed therapy, HER2-expressing CTCs have been followed in patients with breast cancer receiving neoadjuvant trastuzumab, and PD-L1 expression on CTCs has been described in stage IV NSCLC patients receiving nivolumab.[77-79]

Despite a wealth of compelling supportive data, CTCs are rarely used in routine clinical practice. Although technological advances are allowing for improved detection of CTCs, their presence in circulation is vastly outweighed by normal cells, with a signal-to-noise ratio of approximately a billion-to-one.[80] There is also significant variability in the quantities of CTCs among patients of the same cancer type and stage which presents a challenge in developing standardized cut-offs for interpretation. Thus, the greatest utility of CTCs may for monitoring treatment responses by longitudinal sampling within individual patients.

Circulating Nucleic Acids

Circulating *unbound* nucleic acids (ie, cell-free DNA (cfDNA) and RNA (cfRNA)) represent an attractive alternative to CTCs. Circulating nucleic acids were discovered in 1948 and were subsequently found to exist in elevated quantities in patients with cancer.[81,82] Ensuing qualitative analyses of cfDNA in patients with cancer confirmed that a proportion of cfDNA harbors molecular patterns observed in tumor tissue samples, supporting a tumor-cell origin for some cfDNA (defined as "circulating tumor DNA", ctDNA).[83,84] Single-nucleotide variants, insertions, and deletions are the most common alterations used to differentiate ctDNA from cfDNA from other sources, although novel approaches including methylation signatures and bespoke panels personalized to an individual patient's tumor mutations have been used for this purpose.[85,86] Occasionally, the molecular patterns used to distinguish ctDNA from cfDNA are actionable mutations supporting the application of targeted therapies.[87,88]

A relatively unique feature of cfDNA is its short half-life in circulation of approximately 4 minutes to 2 hours.[89] In fact, the kinetics of cfDNA in circulation including its recapitulation by animal models, phases of clearance in humans, and even elimination by hemodialysis have been rather well characterized. This concept of rapid turnover has been leveraged in discussions of using ctDNA as a dynamic marker for monitoring treatment responses.[90] Indeed, in order for a circulating biomarker to be used longitudinally for therapeutic monitoring it must be cleared from circulation rapidly enough to reflect underlying changes in tumor burden.

Circulating Tumor DNA

Early proof-of-concept evidence for the use of ctDNA as a biomarker was provided in a landmark paper by Diehl *et al* in which the authors followed ctDNA in 18 patients with stages II-IV colorectal cancer undergoing multimodal cancer therapy and found that total ctDNA quantities reflected systemic tumor burden.[91] Subsequent work by Dawson *et al* reproduced these findings in patients with metastatic breast cancer and further demonstrated that increases in ctDNA suggestive of treatment failure *preceded* imaging evidence of progression by months in some patients.[92] In metastatic melanoma patients receiving BRAF/MEK-targeted therapy, mutant *BRAF* V600mut ctDNA has been reported to have a sensitivity of 70% and specificity of 100% for detecting progressive disease and in this cohort elevated *BRAF* V600mut ctDNA preceded clinically evident progression in nearly half of cases studied.[93] In fact, having undetectable mutant *BRAF* V600mut ctDNA after 4 weeks of targeted therapy has recently been reported to be significantly associated with improved PFS and OS in patients with advanced melanoma.[94]

Beyond cytotoxic and targeted therapies, multiple potential roles for ctDNA have been proposed in patients receiving immunotherapies. First, ultrasensitive ctDNA assays have been offered as a tool to identify molecular residual disease in patients having undergone definitive treatment which might indicate a need for further interception therapy. Powles *et al* have reported results from a trial of 581 patients with operable urothelial carcinoma (IMvigor010) and showed that patients with detectable ctDNA after surgery had a worse prognosis but achieved a survival benefit from atezolizumab, whereas patients with undetectable ctDNA had an overall better prognosis but experienced no benefit from adjuvant atezolizumab.[95] In the metastatic setting, dynamics of ctDNA levels through therapy (ie, ΔctDNA) have also been associated with disease response in patients receiving checkpoint blockade. In a phase II trial of patients with advanced solid cancer treated with pembrolizumab (INSPIRE), patients with negative (ie, decreasing) ΔctDNA levels through therapy had improved PFS and OS compared with patients with positive ΔctDNA.[96] Nabet *et al* have reported similar observations in patients with stage IV NSCLC and have further created a composite surrogate biomarker integrating pre-treatment parameters (tumor PD-L1 levels, TMB, and circulating CD8 T cells) with early ctDNA kinetics on treatment that could identify patients achieving durable clinical benefit.[97] ΔctDNA has also been shown to be useful in distinguishing progression from pseudoprogression—a transient radiologic increase in tumor size reflective of acute inflammation after immunotherapy rather than tumor growth which can be difficult to identify.[98,99] In a similar vein, ΔctDNA was used alongside radiographic responses in the INSPIRE study to stratify patients into differing immunotherapy sensitivity groups which provided insights into the molecular mechanisms governing treatment response or failure.[99]

Other Cell-Free DNA

Other types of cfDNA have been explored as potentially useful biomarkers. For example, mitochondrial ctDNA has been highlighted for its shorter size, simpler organization, and higher copy number when compared with genomic cfDNA.[100,101] Virally derived sequences are another frequently reported circulating nucleic acid with specific utility in virus-associated malignancies (eg, Epstein-Barr virus in nasopharyngeal cancer, human papillomavirus in cervical and head and neck squamous cell cancers).[102-104] Finally, cfDNA originating from non-malignant cells can also provide information about treatment response in certain clinical scenarios.

Valpione *et al* have recently sequenced T-cell receptor genes from cfDNA in patients with metastatic melanoma undergoing immune checkpoint therapy and identified dynamics that are suggestive of treatment response.[105]

Circulating Tumor RNA

Many different types of ctRNA have been reported to exist including messenger and microRNAs (miRNA), as well as non-coding RNAs such as circular, tRNA-derived, PIWI-interacting, and long non-coding subtypes.[106] Seminal descriptions of stable RNA molecules in circulation were received with some surprise, owing to the belief that serum RNases would render these molecules insufficiently stable for detection.[107-109] Nonetheless, with demonstrated stability in circulation, in addition to reports of tissue-specific expression and frequent dysregulation in cancer, ctRNA have achieved scientific interest as potential circulating biomarkers for monitoring responses to anti-neoplastic therapies.

Perhaps the most widely studied ctRNA are miRNA. Indeed, a plethora of plasma miRNA have been characterized as diagnostic, prognostic, and predictive markers in gastrointestinal, hematologic, lung, prostate, and testicular cancers (among many others).[110-113] As an example, Hansen *et al* have studied miRNA-126 in patients with metastatic colorectal cancer receiving chemotherapy and bevacizumab and have shown that patients with progressive disease tended to have increasing miRNA-126 levels through therapy compared with patients with stable disease or partial/complete responses.[114] Screens for miRNA recapitulating responses to therapy have also been conducted, such as a recent study by Benson *et al* in which the authors identified miRNA-148a as a surrogate for response in patients with recurrent platinum-resistant ovarian cancer receiving carboplatin and decitabine.[115] Finally, as the sensitivity and specificity of single miRNA markers can be suboptimal, there has also been tremendous interest into the possibility of combining multiple circulating miRNA to create a panel with improved operating characteristics.

Clinical Considerations

The relative affordability and ease of detection of circulating nucleic acids have already facilitated their entry into clinical practice. Indeed, the US FDA has already approved the use of several companion diagnostic assays to identify actionable mutations in cfDNA from patients with advanced cancer. Both single gene and next-generation sequencing approaches have been approved for certain indications (eg, EGFR in NSCLC for osimertinib/gefitinib/erlotinib, BRCA1/2 in prostate cancer for rucaparib). Additional efforts are underway to characterize the analytical performance of frequently used assays which should provide fundamental data for the development of clinical guidelines for their routine use.[116]

Exosomes

Extracellular vesicles (EVs) are lipid-bilayer-enclosed, non-replicating endocytic products that are released by a variety of cell types and that have been implicated in diverse biologic functions.[117] Although initially discovered in algae, EVs are now understood to be produced by mammalian cells and are released in vivo in humans.[118-120] Significant quantities of exosomes can be isolated from malignant ascites or pleural effusions in patients with metastatic cancers and EVs have further been detected in saliva, nasal secretions, breast milk, urine, and blood.[121-126] The finding that miRNA expression profiles from isolated circulating exosomes often reflect such profiles from parent tumor tissue samples provided significant credibility to a tumor cell origin for some EVs.[127]

Although not yet routinely used as circulating biomarkers in practice, multiple potential methods for EV analysis have been proposed including measurement of their overall levels and contents.[128] For example, Yu *et al* have monitored plasma exosomes through combination ruxolitinib and erlotinib in stage IV or recurrent *EGFR*-mutated lung adenocarcinomas with erlotinib resistance.[129] Interestingly, the authors observed that the 3 patients with the longest PFS had decreasing exosomal EGFR through therapy, whereas the 13 patients with stable or increasing exosomal EGFR all had shorter PFS. While rather preliminary, these findings highlight the potential for quantitative and qualitative exosomal analysis in prognostication.

The immune checkpoint molecule PD-L1 has been found to exist in circulation on exosomes and changes in response to immunotherapy.[130] Conflicting data exist as to whether increasing or decreasing exosomal PD-L1 through therapy confers a favorable prognosis. Chen *et al* have found that stage III/IV melanoma patients who responded to pembrolizumab tended to have increasing exosomal PD-L1 through therapy which was proposed to be related to heightened IFN-γ-induced PD-L1 expression in the context of T-cell re-invigoration.[131] In contrast, Cordonnier *et al* analyzed exosomal PD-L1 content in patients with stages II-IV melanoma receiving PD-1-, CTLA4-, BRAF-, or MEK-targeted therapies and reported that decreasing exosomal PD-L1 content through therapy is suggestive of treatment response.[132]

As advances are made in characterizing exosomes as circulating biomarkers, existing barriers will have to be overcome to facilitate their integration into clinical practice. New methodologies may be required to replace the rather cumbersome present-day techniques for isolating exosomes from small volume clinical samples. Further, as new techniques with improved detection limits are created, additional efforts may be necessary to distinguish cancer cell-released EV "signal" from background "noise" given the ubiquitous release of EVs from non-neoplastic cells in vivo. Certainly, progress is underway to generate such assays including microfluidic, electrokinetic, and CRISPR-Cas9-based approaches.[133-135]

Metabolomics

Metabolomics refers to the study of the global metabolic profile within a cell or tissue. In contrast to genetic profiling, which reflects biological events that may or may not influence present cellular behavior, metabolic profiling represents current biological state.

Cancer cells rely heavily on aerobic glycolysis and glutaminolysis and this metabolic status is closely linked to aberrant function of oncogenes and tumor suppressor genes.[136-139] Through metabolomics, endogenous metabolites initiating or sustaining oncogenesis ("oncometabolites") were discovered, with 2-hydroxyglutarate being the earliest such molecule described.[140] Nuclear magnetic resonance spectroscopy, gas chromatography mass spectrometry (MS), and liquid chromatography MS are the current technologies used to interrogate the metabolome.[141-143]

Oncometabolites have been offered as biomarkers for cancer diagnosis. Serum levels of lysophosphatidylcholine (LPC) can distinguish the presence of colorectal cancer compared with healthy controls with a 93% specificity and 82% sensitivity.[144] Three other metabolites (histidine, tryptophane, and phenylacetylglutamine) are perturbed during the evolution of precursor lesions into gastric carcinoma and are readily detectable in serum.[145] A number of metabolites have also been characterized in other body fluids from specific tumor types including urine in patients with bladder cancer, and saliva in patients with oral malignancies.[146,147] In the study of pharmacometabolomics, metabolites are used to address questions about pharmacokinetics and therapeutic effect. For example, Backshall *et al* have showed that higher levels of low-density lipoprotein-derived lipids were associated with increased severity of side effects in patients receiving capecitabine for inoperable CRC.[148]

Despite growing enthusiasm about the field of "oncometabolism", a few notable barriers hinder the integration of oncometabolites into clinical practice. Metabolic pathways are highly complex with susceptibility to be influenced by environmental factors, such as diet and gut microbiota.[149] The relatively short half-life of endogenous metabolites, as well as their dependence on environmental factors, makes it imperative that consistency in sampling is sought. Further globally accepted validation studies in large cohorts of patients are still needed. Due to the complexity of metabolic networks, it is widely believed that panels of metabolites are a more representative biomarker of the metabolic state, rather than isolated single metabolites. However, panel analysis can lead to generation of large databases which can be challenging to interpret. Thus, metabolomics are not yet widely implemented in clinical spaces.

Conclusions and Future Directions

The rapid determination of treatment response has been a longstanding priority in oncology. Prompt identification of disease relapse after definitive treatment may indicate the need for interception therapy whereas, in the metastatic setting, early recognition of treatment failure may provide patients an opportunity to move on to more efficacious treatments and limits exposures to toxicities. Circulating biomarkers such as non-specific markers of disease burden and tumor markers have been used in the clinical setting to aid treatment monitoring. With notable exceptions such as PSA, the majority of these markers have not replaced standard radiological imaging for decision-making. Other circulating biomarkers reviewed in this manuscript are actively under investigation, each with particular advantages and disadvantages (Fig. 2).

As future work continues, it will be essential that the performance of these biomarkers is well-characterized prior to their integration into routine clinical practice. Indeed, overreliance on poorly understood biomarkers poses significant risks to patients including increased frequency of interactions with the healthcare system, financial cost, stress from false positives/negatives, and even the possibility of abandoning efficacious treatments prematurely or exhausting lines of therapy. Additional careful consideration will need to be given to the role of proprietary industry-generated assays for biomarker detection. Finally, and ideally, the integration of these biomarkers into clinical practice would be justified by clinical data showing that their use in identifying subclinical disease progression has an actual effect on improving patient-centered outcomes.

Certainly, there are plentiful opportunities for the development of circulating biomarkers that meet these high

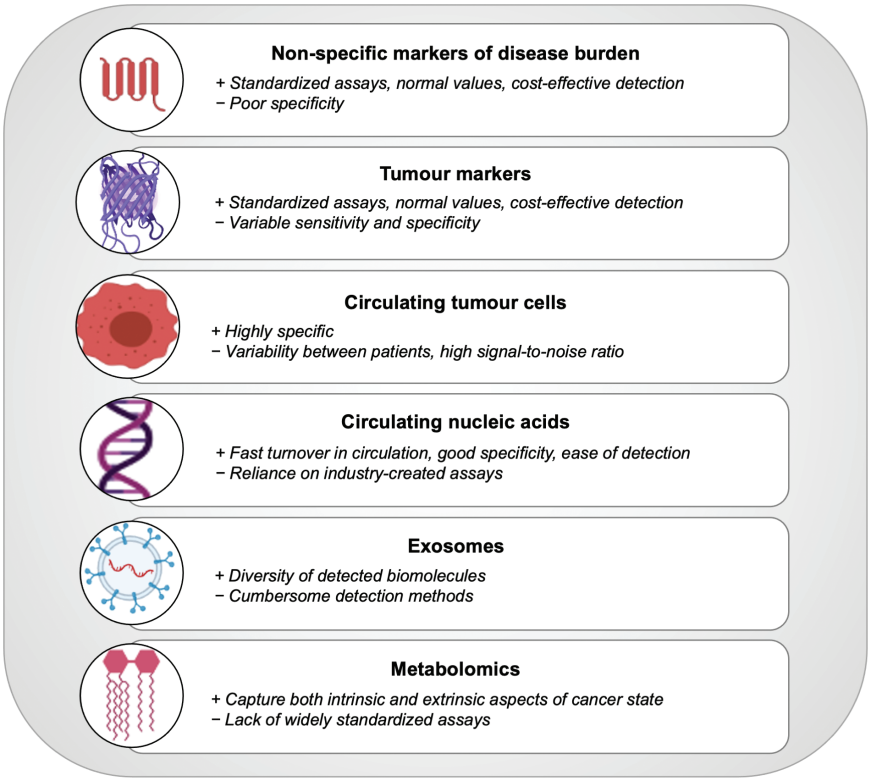

Figure 2. Strengths and weaknesses of circulating biomarkers for monitoring treatment response.

standards. The diverse cancer secretome provides no shortage of circulating macromolecules that can be evaluated individually or in combination through multiplexed approaches as well as machine learning algorithms to create composite biomarkers. Beyond blood-based biomarkers, assessment of additional non-invasive biomarkers, for instance, radiomic evaluation of quantitative features on routine radiological imaging, as well as interrogation of other body substances such as urine or stool for microbiome analysis, may offer a holistic understanding of the interactions between host, tumor, and the microenvironment.[150] With efforts to this end already underway, and a wealth of further possibilities, the field of circulating biomarkers for disease monitoring appears promising.

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Conflict of Interest

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(C/A) consulting/advisory relationship; (RF) research funding; (E) employment; (ET) expert testimony; (H) honoraria received; (OI) ownership interests; (IP) intellectual property rights/inventor/patent holder; (SAB) scientific advisory board.

Author Contributions

Conception/design: All authors. Manuscript writing: All authors. Final approval of manuscript: All authors.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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