Review Article

Personalized medicine: From diagnostic to adaptive

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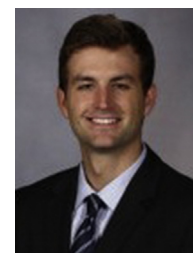
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ABSTRACT

Personalized therapy has made great strides but suffers from the lack of companion diagnostics. With the dawn of extracellular vesicle (EV) based liquid biopsies fast approaching, this article proposes a novel approach to cancer treatment — *adaptive* therapy. Already being implemented in the field of radiation oncology, adaptive radiation therapy utilizes cutting-edge imaging techniques as a viable means to monitor a patient's tumor throughout the entire treatment cycle by adapting the dosage and alignment to match the dynamic tumor. Through an EV liquid biopsy, medical oncologists will also soon have the means to continuously monitor a patient's tumor as it changes over time. With this information, physicians will be able to "adapt" pre-planned therapies concurrently with the fluctuating tumor environment, thus creating a more precise personalized medicine. In this article, a theory for adaptive medicine and the current state of the field with an outlook on future challenges are discussed.

Current diagnostics in personalized medicine

Personalized cancer therapy — a significant milestone for modern medicine — involves developing treatment strategies centered around characteristics that make each individual patient's cancer unique [1]. Over the past few decades, fundamental cancer biology research has uncovered many of the cellular and molecular mechanisms involved in tumor formation, proliferation and metastasis. Underlying these mechanisms are distinguishing features in many different forms including genetic mutations [2—4], chromosomal abnormalities [5], epigenetic changes [6,7] and dynamic tumor-

host interactions [8]. While these various features contribute to cancer development, they also create "weaknesses" or targets that researchers can develop drugs against. Perhaps the poster child for this movement, and a drug that exemplifies the potential involved in developing personalized therapies, is Imatinib [9]. Imatinib, a drug for chronic myelogenous leukemia (CML), specifically inactivates a mutant tyrosine kinase encoded by the fusion of two genes: *BCR-ABL*. This genetic construct is created in cells after an abnormal chromosomal translocation and results in cancerous transformation and uncontrolled growth [10]. Thus, a drug targeted to the product of this gene should selectively kill leukemia cells and spare

healthy cells. In fact, it does just that — showing great efficacy and low toxicity — setting the bar for personalized cancer therapy [9]. Since the development of Imatinib many other targeted therapies have reached FDA approval, such as the *BRAF* inhibitor Vemurafenib for the treatment of metastatic melanoma and the *EGFR* inhibitor Cetuximab for the treatment of metastatic colorectal cancer [11–13], non-small cell lung cancer [11] and head and neck cancer [13]. Even current immunotherapies, such as PD-1 blocking antibodies, are now being applied in a personalized way — with clinicians preferentially administering Pembrolizumab to patients whose tumors have detectable levels of PD-L1 expressed [14–16].

Although some of these targeted therapy agents such as Imatinib have seen success, most other promising drugs have produced disappointing results. Our knowledge base for molecular targets has grown substantially over recent years [17,18], but the concurrent drug development has not kept pace. The leading presumptive reason is that diagnostics for patient stratification and monitoring treatment response are lacking. In the vast majority of patients, tumors are biopsied through tissue sampling and subsequent genomic characterization. This technique, however, is inherently flawed and often difficult to perform. Through both a surgical or needle approach, the protocol for acquiring a tissue sample is costly, invasive, painful and can pose a risk to the patient [19–21]. Due to the heterogeneous nature of tumors, biopsies also suffer from sample bias with as many as two-thirds of mutations from a single biopsy unable to be detected throughout the rest of the sampled region in the same tumor [22,23]. Moreover, collecting tissue samples from certain cancer types remains technically challenging and the biopsy frequently fails due to an inadequate amount of tissue for genetic testing [24,25]. In the case of endometrial cancer, as many as 26.2% of tissue biopsies produce inadequate samples [26]. Furthermore, one study showed 31% of advanced non-small cell lung cancer (NSCLC) patients did not have accessible tissue [27]. Even when a sample can be collected, clinicians must be wary as a commonly used preservative, formalin, causes DNA fragmentation and C > T transitions through the deamination of cytosine, potentially leading to false positives. In fact, one report identified a false positive NRAS G12D mutation in a melanoma sample due to these fixation artifacts [28].

As mentioned, tumors display spatial heterogeneity — one area may be genetically/phenotypically distinct from another — but, perhaps even more importantly, tumors display temporal heterogeneity [29]. Acquired drug resistance is common over the course of an extended treatment [30], thus there is desperate need for a tool to monitor tumor evolution and predict arising resistance before tumor resurgence. Our current diagnostic methods of tissue sampling only inform us of the genotype/phenotype at the particular time point at which the sample is retrieved. Developing a personalized therapy to combat a dynamic tumor based on a static diagnostic is obviously problematic. To illustrate, approximately 25% of human breast cancers overexpress the HER2 proto-oncogene, a common personalized therapy drug target [31]. A significant fraction of patients being treated with the monoclonal antibody against HER2 (Trastuzumab), however, eventually relapse or develop progressive disease [31]. In another

example, 38% of colorectal cancers treated with anti-*EGFR* therapy developed KRAS mutations as rapidly as 5 months after treatment onset [32]. Even checkpoint inhibitors (anti-PD-1, anti-PD-L1, anti-CTLA4), which are revolutionizing treatment options and expectations for melanoma patients, are victims to tumor resistance [33].

Although traditional biomarkers are used widely and often successfully in conjunction with some targeted therapies, our capabilities of developing truly personalized medicines are limited by these static biomarkers. Although technically challenging, a liquid biopsy with the potential to both diagnose specific tumor mutations and monitor treatment response will allow clinicians to treat patients in a more precise manner. This review will focus on extracellular vesicles — nano-sized, membrane-bound particles actively released by cells and found ubiquitously in body fluids [34] — as a liquid biopsy source that will enable clinicians to implement an *adaptive* approach to various cancer therapies.

A better biopsy

A liquid biopsy is obtained from samples of body fluids (plasma, urine, cerebrospinal fluid, saliva) and offers a unique opportunity to obtain cancer-derived materials in a non-invasive manner. Consequently, it is practical for real-time patient monitoring and analyzing treatment response. The three leading cancer-derived materials for this technology are circulating tumor cells (CTCs) [35], cell-free DNA (cfDNA) [36], and extracellular vesicles (EVs) [37]. While all three have shown some promise, many technical hurdles still remain. For instance, while CTCs have been detected in breast, prostate, lung and colorectal cancer [38–40], they are often hard to discriminate from other circulating cells. CTCs are defined as cytokeratin positive, epithelial cell adhesion molecule (EpCAM) positive and CD45 negative, but unfortunately, these markers are not always expressed — leading to false negatives [41]. Furthermore, CTCs can acquire "private" genetic mutations that differ from the genome of the tumor [42]. On the other hand, cfDNA biopsies suffer from low copy number of mutant alleles, and median half-life of the cfDNA in circulation ranges from only 15 min to a few hours [43,44]. While both of these fields undoubtedly show promise and deserve to be explored, this review will focus on the third method: extracellular vesicles.

With respect to nomenclature, the definition of extracellular vesicles (EVs) includes a broad spectrum of nanoparticles including classic exosomes and microvesicles, as well as other shed vesicles and membrane particles. Exosomes are defined as 30–150 nm endosomal-derived vesicles, while microvesicles vary largely in size (50–1000 nm) and are generated from budding of the plasma membrane [34,45]. The field has quite recently determined the term "EV" to be appropriate in discussing these particles because it is difficult to discriminate between the different populations.

EVs are actively released from a variety of cells and carry proteins, nucleic acids and lipids that are involved in mediating intercellular communication to regulate a number of physiological roles in health and disease [45–47]. Diverse functions have been uncovered, primarily dependent upon

the cell of origin. For instance, T cells utilize EVs for unidirectional modulation of antigen-presenting cells [48]; EVs of neural origin communicate with glial cells [49]; EVs present in synovial fluid contribute to joint development [50]; and stem cells even secrete EVs for interaction with injured tissue [51]. Cancer cells, however, can override the normal cellular mechanism for producing these vesicles and generate EVs that stimulate growth/proliferation [52], angiogenesis [53,54], epigenetic reprogramming [55], generation of pre-metastatic niches [56,57], and metastasis [54,58].

The large role that EVs play in tumor development suggests that they may be an immense source of untapped information with great potential as cancer biomarkers in personalized medicine. Other characteristics imply this may be the case as well. Firstly, the cargo transferred by EVs is highly specific and represents the state and composition of its parental cell [59]. Secondly, the idea that EVs are released in subsets [60], with distinctive profiles and functions, suggests that they carry "packets" of multidimensional data that can be more informative than a single molecule biomarker. The number of EVs in circulation is also quite astounding, with studies confirming up to 10^{12} EV/mL of blood [61] (CTCs, for instance, are found at 1–10/mL blood) [62]. Research has also shown that EV number changes with both tumor development and regression [63,64]. For example, Kim, et al. showed an increasing number of EVs with gastric cancer progression [63], and Baran et al. took this even further and correlated the stage of gastric cancer development with the number of EVs found in circulation [64].

The structure of EVs, specifically the lipid bilayer, also confers advantages. The RNA or protein cargo contained within EV subsets is protected from degradation by circulating nucleases and proteinases. Consequently, biomarkers that have relatively low expression are much easier to be detected than their soluble counterparts. For instance, the potential prostate mRNA biomarkers PCA3 and TMPRSS2 appear in EVs but are not easily detectable in body fluids [65]. In fact, it has been shown that the majority of miRNA detectable in serum and saliva are contained within EVs [66]. The EV lipid bilayer also serves to reduce sample complexity compared to body fluids. When 22 proteins make up 99% of blood plasma, it is understandably difficult to detect less abundant soluble proteins [67]. For example, prostate-specific antigen, the classic prostate cancer biomarker, possesses higher specificity to the disease when detected on EVs rather than the total amount in serum or plasma [68,69].

By now, there has been an immense effort to turn the extracellular vesicle into a cancer biomarker. Some of these studies have been summarized in [Table 1]. Our interest, however, is in how these biomarkers can revolutionize personalized medicine. In fact, EVs can be exploited as a companion diagnostic to assist in identifying patients who will benefit most from certain drugs [70]. Kahlert et al. showed that mutated KRAS in EVs is associated with poor therapeutic response which opens the door for a companion diagnostic for Cetuximab [71,72]. Thakur et al. further confirmed this work and also showed that BRAFV600E in EVs can serve as a companion diagnostic for treating melanoma with Vemurafenib [73].

The cutting edge

The last few years has seen a surge of new data detailing the profound potential of extracellular vesicles as diagnostic, prognostic, predictive, and treatment monitoring liquid biopsies [see Table 1]. For pancreatic cancer, in which early detection is vital, five separate studies have demonstrated that the presence of certain EVs are strongly correlated with disease. Que et al., for instance, determined that levels of exosomal miR-17-5p and miR-21 were significantly elevated in the serum of pancreatic cancer patients. Further, levels of miR-17-5p were correlated with both metastasis and advanced stage of the cancer [77]. Similarly, Madhaven, et al. discovered that a panel of 5 proteins and 4miRNA (CD44v6, Tspan8, EpCAM, MET, CD104, miR-1246, miR-4644, miR-3976, and miR-4306) could detect pancreatic cancer with a sensitivity of 100% and a specificity of 80% [77].

EV diagnostic assays are being explored in relation to other cancers as well. In one lung cancer study, a significant difference was found in total exosome levels between lung adenocarcinoma patients and controls [80]. CD151 has also been established as an individual marker for separating squamous cell and small cell lung cancer from controls [82]. Moreover, Non-small cell lung cancer (NSCLC) patients have a remarkably different EV repertoire than healthy patients. One study demonstrated this by creating a 30-marker model to separate NSCLC patients from healthy controls. This model has a sensitivity of 75% and a specificity of 76%; it also classifies patients with 75.3% accuracy [85].

There is a remarkable need for improved diagnostics in prostate cancer patients as well. Standard tests such as prostate-specific antigen (PSA) measurement have poor specificity (33%) resulting in an alarming number of false positive reports, invasive biopsies, and harmful procedures. To combat this, eleven studies have investigated the difference in EV repertoire between prostate cancer patients and healthy controls in the past five years alone. Park et al., for one, showed that plasma levels of PSMA-positive EVs could significantly delineate patients with BPH and low-, intermediate-, and high-risk prostate cancer (21.9, 43.4, 49.2, 59.9 ng/mL, $p < 0.001$). ROC curve analysis indicated that PSMA + EVs could detect prostate cancer from BPH with an AUC of 0.943 [92]. Plasma is not the only source of useful EVs, however. Koppers-Lalic et al. investigated differential expression of miRNA in the urine of prostate cancer patients and found that miRNA isoforms with 3' end modifications were highly discriminatory between samples from control men and prostate cancer patients. A diagnostic panel using isoforms of miR-21, miR-204, and miR-375 detected within EVs was shown to have a sensitivity of 72.9% and specificity of 88%, superior than the commonly used PSA measurement [95].

Breast cancer, the most prevalent cancer diagnosis for women in the United States, is also a prime target for EV diagnostic assays. Mammography, the current gold standard, has a decent sensitivity (75–90%) and specificity (90–95%), but only boasts a 20% positive predictive value for women under age 50. Many studies have aimed to address this issue. For

Table 1 Examples of cargo associated with extracellular vesicles as potential markers for personalized medicine.

Cancer Type	Biomarker	Type	Source	Application
Pancreatic	GPC-1	Protein	Serum	Diagnosis/Prognosis [74]
	EGFR	Protein	Plasma	Diagnosis [75]
	miR-17-5p, miR21	miRNA	Serum	Diagnosis [76]
	KRAS	DNA	Serum	Diagnosis/Prognosis [71]
	5 proteins, 4miRNAs	Combination	Serum	Diagnostic [77]
	11 miRNA	miRNA	Plasma	Diangostic [78]
Lung	EGFR	Protein	Serum	Prognosis [72]
	EDIL3	Protein	*ex vivo*	Prognosis [79]
	EpCAM + EV number	N/A	Plasma	Screening/Prognosis [80]
	LRG1	Protein	Urine	Diagnosis [81]
	CD151	Protein	Plasma	Diagnosis [82]
	EGFR/phospho-EGFR	Protein	*ex vivo*	Monitoring [83]
	Integrins α6β4, α6β1	Protein	Plasma	Metastatic Pattern [84]
	30 proteins	Protein	Plasma	Diagnosis [85]
	miR-486, miR30d, miR499	miRNA	Serum	Diagnosis/Prognosis [86]
	12 miRNAs	miRNA	Plasma	Screening/Prognosis [80]
	SRGN, TPM3, THBS1, HUWE1	Protein	Plasma	Diagnosis [87]
Prostate	GPC-1	Protein	Plasma, Urine	Diagnosis [88]
	Survivin	Protein	Plasma/Serum	Diagnosis/Monitoring [89]
	PSA	Protein	Plasma	Diagnosis/Prognosis [69]
	CDH3	Protein	Urine	Prognosis [90]
	PSA	Protein	Urine	Diagnosis [68]
	β-Catenin	Protein	Urine	Screening [91]
	PCA-3, TMPRSS2:ERG	Protein	Urine	Diagnosis/Monitoring [65]
	P-glycoprotein	Protein	Serum	Monitoring Resistance [92]
	PSMA	Protein	Plasma	Diagnosis/Prognosis [93]
	ITGB4, VCL	Protein	*ex vivo*	Prognosis/Monitoring [94]
	isomiRs of miR-21, miR-204, miR-375	miRNA	Urine	Diagnosis [95]
Breast	Del-1	Protein	Plasma	Diagnosis [96]
	Fibronectin	Protein	Plasma	Diagnosis [97]
	EDIL3	Protein	Plasma	Prognosis [79]
	CD24, EpCAM	Protein	Serum	Diagnosis [98]
	BCRP	Protein	Plasma	Prognosis/Monitoring [99]
	miR200a, miR200c, miR-205	miRNA	Serum	Diagnosis [98]
	miR-21	miRNA	Serum	Prognosis [100]
	miR-373	miRNA	Serum	Diagnosis/Prognosis [101]
	miR-134	miRNA	*ex vivo*	Prognosis [102]
Colorectal	Claudin-3	Protein	Ascites	Diagnosis [103]
	HGS	Protein	Plasma	Prognosis [104]
	Hsp60	Protein	Plasma	Monitoring/Prognosis [105]
	EGFR/phospho-EGFR	Protein	*ex vivo*	Monitoring [83]
	BRAF	DNA	Serum	Diagnosis [73]
	7miRNAs	miRNA	Serum	Diagnosis [103]
	miR-200c, miR-141	miRNA	*ex vivo*	Monitoring [106]
	KRTAP5-4, MAGEA3, BCAR4	miRNA	Serum	Diagnosis [107]
	miR-200c, miR141	miRNA	*ex vivo*	Monitoring [106]
	miR-19a	miRNA	Serum	Prognosis [108]
Melanoma	Caveolin-1, CD63	Protein	Plasma	Prognosis [109]
	miR-125b	miRNA	Serum	Prognosis/Monitoring [110]
	miR-146a	miRNA	Serum	Diagnosis [111]
Glioblastoma	EGFRvIII	Protein	Serum	Prognosis [112]
	EGFR, EGFRvIII, PDPN, IDH1R132H	Protein	Plasma	Monitoring [113]
	Annexin V + EV Number	N/A	Serum	Prognosis/Monitoring [114]
	MMP9	Protein	Serum	Diagnosis
	miR-21	miRNA	Serum	Diagnosis/Prognosis [115]
	miR-21	miRNA	CSF	Diagnosis [116]
	9 miRNAs	miRNA	CSF	Diagnosis [117]

Table 1 – (continued)

Cancer Type	Biomarker	Type	Source	Application
Medulloblastoma	ErbB2/HER2	Protein	Serum	Diagnosis [118]
Ovarian	Claudin -4	Protein	Plasma	Diagnosis [119]
	CD24, EpCAM	Protein	Ascites	Diagnosis [120]
	L1CAM, CD24, ADAM10, EMMPRIN	Protein	Serum	Diagnosis/Prognosis [121]
	TGF-beta1, MAGE3/6	Protein	Plasma	Prognosis/Monitoring [122]
	12 miRNAs	miRNA	Serum	Diagnosis [123]
	8 miRNAs	miRNA	Serum	Diagnosis [124]
Bladder	Periostin	Protein	Urine	Diagnosis/Prognosis [125]
	HOTAIR	lncRNA	Urine	Diagnosis/Prognosis [126]
Multiple Myeloma Gastric	CD44	Protein	Serum	Prognosis [127]
	miR-21, miR-1225-5p	miRNA	Ascites	Diagnosis/Prognosis [128]
	LINC00152	lncRNA	Plasma	Diagnosis [129]
Esophageal	10 miRNAs	miRNA	Serum	Diagnosis [130]
Thyroid	miR-146b, miR-222	miRNA	*ex vivo*	Diagnosis [131]
Liver	miR-718	miRNA	Serum	Prognosis [132]
DLBCL	CD63, Alix, TSG101, CD81	Protein	*ex vivo*	Diagnosis [133]
HNSCC	miR-486-5p, miR-486-3p, miR-10b-5p	miRNA	*ex vivo*	Diagnosis [134]
Osteosarcoma	miR-25-3p	miRNA	Serum	Diagnosis/Prognosis [135]

Abbreviations: DLBCL: Diffuse Large B-Cell Lymphoma; HNSCC: Head and Neck Squamous Cell Carcinoma.

instance, Moon, et al. showed a correlation with Del-1+ EVs to early-stage breast cancer, with a published sensitivity and specificity of 95% and 86%, respectively [96]. Another study published by the same author found a diagnostic candidate in fibronectin [97]. Lee et al. sought to investigate the role of EDIL3 on EVs in breast cancer patients. This study demonstrated that EDIL3 is involved in invasion and acceleration of lung metastasis in vivo. Furthermore, it also showed that levels of EDIL3 were significantly upregulated on breast cancer EVs. Thus, they not only discovered a new therapeutic target, but also a potential diagnostic target [79].

The diagnostic uses of EVs are far-reaching and applicable to many other cancer subtypes, as demonstrated in [Table 1]. It is important, however, to note that the development of a liquid biopsy enables the field to do much more than simply detect disease earlier. Many of these studies hold prognostic value as well. For instance, one recent study demonstrated that miR-718 was differentially expressed in the serum exosomes of hepatocellular carcinoma patients with recurrence after liver transplant than those without recurrence. Decreased expression of miR-718 was also associated with decreased tumor aggressiveness [132].

EV liquid biopsies will also soon assist clinicians in treatment decisions. Strong data suggests that these assays can predict the therapeutic response of various chemotherapies. Kahlert et al., in a breakthrough study, found evidence that dsDNA fragments contained within EVs may be as long as 10-kb. With this new data, they were able to show that EV-derived DNA carries mutations identical to their parental cells or tumors. Specifically, they detected KRAS mutations in the EVs of pancreatic cancer patients which correlated to the primary tumor [71]. This data suggests that it may be viable to base treatment decisions, specifically whether a patient qualifies for cetuximab therapy, based on EV genomics. Another study investigated docetaxel resistance in prostate cancer patients. Docetaxel is used as the first-line

chemotherapy for castration-resistant prostate cancer (CRPC), but resistance is prevalent due to acquired induction of P-glycoprotein encoded by the multidrug resistance protein 1 (MDR1) gene. In resistant patients, a new taxane – cabazitaxel – is being used due to its poor affinity for P-glycoprotein. Researchers have demonstrated a significantly higher P-glycoprotein level in serum EVs from docetaxel-resistant patients when compared to docetaxel-susceptible patients. Therefore, this assay has the potential to be used as a guide for the selection of an appropriate taxoid in patients with CRPC [92]. Lastly, Thakur, et al., showed that BRAFV600E mutations can be detected within EV dsDNA, suggesting a screen for melanoma patients who are likely to respond to vemurafenib [73].

Perhaps the most important use of any liquid biopsy, including one based upon EVs, is the ability to monitor response to treatment. This, however, is unfortunately the least investigated use for this new technology. While only ten of the 71 studies exploring EV biomarkers in the last few years has explored treatment monitoring, there have been remarkable findings. Yuwen et al., for example, demonstrated that the expression of exosomal miR-146a-5p in NSCLC patients decreased gradually in concordance with the development of resistance to cisplatin chemotherapy. Real-time monitoring of cisplatin resistance may be possible with an assay such as this, saving patients both time and money. In another example, Shao, et al. investigated the use of microvesicles for monitoring the therapeutic response of glioblastomas to temozolomide (TMZ) and concomitant radiation therapy. They were able to prove a strong negative correlation between successful treatment response and glioblastoma-specific microvesicles (EGFR, EGFRvIII, PDPN, IDH1R132H positive-EVs). Lastly, Melo, et al. determined that serum levels of Glypican-1 (GPC1) positive EVs correlated with tumor burden and were able to detect both early and late stage pancreatic cancer with absolute specificity and sensitivity.

Most importantly, the authors investigated GPC1+ EV levels one week after tumor resection. They found that the 50% of patients with the greatest decrease in GPC1+ EVs had an average lifespan of approximately twice as long when compared to the 50% of patients with a lesser decrease [74]. This suggests the possibility of developing a short-term treatment monitoring assay. The field, however, still lacks data regarding using liquid biopsies to monitor treatment response, as seen in [Table 1].

Transitioning to *adaptive* therapies

The development of an extracellular vesicle liquid biopsy gives us not only the potential to advance diagnostics, but also to usher in an era of adaptive medicine. The phrase "adaptive medicine" carries many different meanings depending on the scientist or clinician you have engaged in conversation. The definition used in this article, however, bridges all medical fields: modifying the original treatment plan throughout the treatment course using routine liquid biopsies to monitor a patient's response and make clinical decisions appropriate for each specific patient. For instance, are there specific EV subsets that correlate with patients who will successfully respond to treatment? Does emerging treatment resistance change these subsets? If so, we can modify each patient's treatment plan in real-time — preventing tumor progression and saving patients large amounts of both time and money. One recent study showed that treatment with the EGFR inhibitor, Cetuximab, triggered a burst of exosome-like EVs containing specific markers: EGFR, p-EGFR, and genomic DNA (gDNA) [136]. The same study concluded that "targeted agents may induce cancer cells to change the EV emission profiles reflective of drug-related therapeutic stress," which was further verified by another study examining EVs after Cetuximab treatment [83]. It is, therefore, not unreasonable to hypothesize that these EV emission profiles can be characterized and used to determine the efficacy of particular treatments in different patients.

This adaptive concept is not an entirely new one. It has been implemented and successfully utilized in radiation oncology in recent years. Adaptive radiation therapy (ART) is state-of-the-art in optimizing precision and accuracy of dose delivery. First introduced by Yan et al., "to minimize the deleterious effects of setup variation on each individual patient," ART uses cutting edge imaging techniques to modify initial treatment plans — accommodating changing anatomy (i.e. shrinking tumor) or changing environment (i.e. tumor hypoxia), so that the therapy will remain accurate and efficacious to each patient throughout his/her treatment course [137]. Ahn et al. demonstrated a benefit in 65% of head and neck cancer patients resulting from adaptive planning that corrected inadequate dose to tumor or excess dose to surrounding organs [138]. Murthy et al. also concluded that using an adaptive approach for bladder cancer was clinically feasible and produced good oncological outcomes [139].

Recently, positron emission tomography (PET), an imaging tool that can be used to illuminate a tumors metabolic activity, stage and progression, has been investigated as a means to introduce an adaptive approach to neoadjuvant chemotherapy. The goal of this technique is to distinguish between individuals who will be good responders to therapy (PET negative) or poor responders (PET-positive). With this information, clinicians can either change therapies or adjust the dose given to match each patient's needs [140]. In fact, Chaft, et al. investigated adaptive neoadjuvant chemotherapy in non-small cell lung cancers and concluded that using PET/CT scans to assess response and change preoperative chemotherapy in nonresponding patients can improve long-term outcomes [141].

The adaptive concept, however, should not just be limited to radiation and neoadjuvant therapy. With emerging liquid biopsies, oncologists administering other cancer treatments will soon have the means to monitor their patients in real time. Immunotherapists will have a better understanding of the tumor microenvironment when selecting checkpoint inhibitors, and clinicians administering chemotherapies will see resistance emerging in real-time. Furthermore, another remarkable theoretical treatment model proposed by Enriquez-Navas et al. would stand to benefit [142]. They proposed an adaptive treatment strategy that refutes the widely accepted maxim that chemotherapies should be applied at the maximum tolerated dose. This novel approach applies limited, short bursts of therapy with the goal of maintaining a population of treatment-susceptible cells. When the tumor is no longer exposed to the chemotherapy, this controllable population suppresses the proliferation of drug-resistant cells due to its fitness advantage in normal conditions. This method proved quite effective for paclitaxel treatment in two different mouse models, but has not yet been implemented in human trials. This, and most other cancer treatment options, would benefit enormously by liquid biopsy technology enabling simple, efficient tumor monitoring.

Challenges ahead

While extracellular vesicles are currently used extensively in research laboratories across the globe, many challenges remain before reliable EV diagnostics enter the clinic. The most formidable problem, undoubtedly, concerns isolation of EVs. There is currently no standardized protocol for isolation — with published techniques including differential centrifugation [143], density gradient centrifugation [144], sequential membrane filtration [145], size exclusion chromatography [146], microfluidics [147], nanoshearing [148] and various kits from biotechnology companies. Differential centrifugation is the most widely used procedure for EV isolation, but it is low-throughput, low-yield and operator dependent. Additionally, samples of plasma often co-precipitate lipoproteins, further convoluting results and rendering this technique incompatible with clinical practice.

A huge number of potential EV biomarkers have been published in just the last few years — a promising start [Table 1]. This, however, creates a problem in itself. The vast majority of these samples are derived from small-sample studies or *ex vivo*, therefore requiring validation through larger and more rigorous studies. With these bigger undertakings, more

efficient characterization of EV profiles is needed. Mass spectrometry and next-generation RNA sequencing are commonly used for bulk analyses. However, sorting EVs on a single-particle basis is needed in order to distinguish between EV subsets with distinct origins that have distinctive profiles and functions. A novel method of high resolution flow cytometry called nanoFACS has allowed for advancement of analysis and sorting of individual EVs from body fluids [149]. This method holds great promise for the development of the EV liquid biopsy, because it opens the possibility to analyze characteristics of EV subsets (tumor-derived EVs for example), rather than bulk plasma-derived EVs. Lastly, basic properties of extracellular vesicle biology are insufficient and further information is needed on synthesis, secretion and storage of these vesicles.

Conclusion

It is now widely accepted that extracellular vesicles play an integral role in cellular communication and tumor progression with compositions that correspond to the parental cell identity and state. With many advantages, extracellular vesicles are a promising source of biomarkers for which to develop liquid biopsies. After challenges such as isolation of extracellular vesicles are overcome, clinicians will have the means to adapt pre-planned therapies to correspond with rapidly changing tumors with high sensitivity and accuracy.

Conflicts of interest

The authors declare no conflicts of interest.

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