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REVIEW

Incorporating blood-based liquid biopsy information into cancer staging: time for a TNMB system?

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Tissue biopsy is the standard diagnostic procedure for cancer. Biopsy may also provide material for genotyping, which can assist in the diagnosis and selection of targeted therapies but may fall short in cases of inadequate sampling, particularly from highly heterogeneous tumors. Traditional tissue biopsy suffers greater limitations in its prognostic capability over the course of disease, most obviously as an invasive procedure with potential complications, but also with respect to probable tumor clonal evolution and metastasis over time from initial biopsy evaluation. Recent work highlights circulating tumor DNA (ctDNA) present in the blood as a supplemental, or perhaps an alternative, source of DNA to identify the clinically relevant cancer mutational landscape. Indeed, this noninvasive approach may facilitate repeated monitoring of disease progression and treatment response, serving as a means to guide targeted therapies based on detected actionable mutations in patients with advanced or metastatic solid tumors. Notably, ctDNA is heralding a revolution in the range of genomic profiling and molecular mechanisms to be utilized in the battle against cancer. This review will discuss the biology of ctDNA, current methods of detection and potential applications of this information in tumor diagnosis, treatment, and disease prognosis. Conventional classification of tumors to describe cancer stage follow the TNM notation system, heavily weighting local tumor extent (T), lymph node invasion (N), and detectable metastasis (M). With recent advancements in genomics and bioinformatics, it is conceivable that routine analysis of ctDNA from liquid biopsy (B) may make cancer diagnosis, treatment, and prognosis more accurate for individual patients. We put forward the futuristic concept of TNMB tumor classification, opening a new horizon for precision medicine with the hope of creating better outcomes for cancer patients.

Key words: liquid biopsy, noninvasive, circulating tumor DNA, cancer, cancer staging

Introduction

Malignant tumors are highly heterogeneous at multiple levels [1, 2]. Histologically, tumor tissues may exhibit remarkable variation in morphology and cellular composition within different regions of the same tumor, as well as among different tumors from the same primary site [3, 4]. In the case of metastatic cancer, metastases to regional lymph nodes and at distant sites present further divergence [5]. These heterogeneities may not be fully represented in morphology-based pathological classifications from biopsy of the primary tumor site at diagnosis. More recently, genomic analyses along with molecular characterization of cancers have helped reveal the foundation for these differences [6–8]. Complex relationships with the local tumor environment, particularly immune cells, may alter disease progression. Indeed, there is little doubt that cancer displays dynamic evolution

during disease progression. Both intrinsic and extrinsic forces act to drive cancer cells to move—to metastasize—transforming cancer to a systemic disease. While initial biopsy diagnosis and primary tumor site offer critical information for cancer treatment, new methodologies offering diagnostic sensitivity and longitudinal assessment are needed.

Cancer management relies on staging at initial cancer diagnosis. Conventional staging of malignant tumors follows the TNM notation system, encompassing tumor extent (T), lymph node invasion (N), and detectable metastasis (M) [9]. New understanding of genetic and molecular drivers for cancer has led to development of drugs targeting these mutational events [10], and targeted therapy has provided increasing numbers of success stories [11, 12]. Too often, however, these responses are short-lived, and it is understood that a major limitation is the heterogeneous, fundamentally dynamic, and inherently systemic nature of cancer. Considering these barriers, by improving disease characterization—both initially and over cancer progression—the more precisely targeted therapy can be applied. Insight into the systemic nature of cancer has emerged from the study of circulating tumor cells (CTCs), recognizing that cancer cells and their by-products can be detected circulating in blood [13]. Interest in human genomics has driven rapid advances in DNA and RNA sequencing technologies. Streamlined and highly sensitive next-generation sequencing (NGS) has facilitated analysis of cancer mutations in blood [14, 15]. This expanding capacity to detect cancer-specific mutations in circulation, particularly using a noninvasive procedure that enables resampling over time, provides tremendous potential for cancer diagnosis, prognosis, and actionable treatment [16]. Improved characterization of CTCs [17] and cancer-derived DNA [18], RNA [19], and protein-based [20] markers offers additional targets for development of therapeutics. Although not a new concept, a shift toward routine implementation of liquid biopsy as a cancer diagnostic and prognostic tool stands to benefit patients by providing a noninvasive means to detect clinically actionable genetic alterations, and importantly, to monitor disease progression and treatment resistance in real time (Figure 1).

The clinical applications of circulating tumor DNA (ctDNA) as a 'liquid biopsy' have been actively investigated in recent years. Advancements in NGS technologies are making DNA-based liquid biopsy feasible to gain understanding of cancer mechanisms and to predict drug responses [21, 22]. Recent studies support the role of ctDNA as a clinically valuable readout for prognostic staging at diagnosis and monitoring over time [23]. In this new era of precision medicine, we propose that there is diagnostic, therapeutic, and prognostic value to implementing blood-based ctDNA testing concurrent with updating the current TNM system of cancer staging to include this information in a modified system, we suggest as 'TNMB' staging.

The history of ctDNA and its utility in cancer

The first evidence of cell-free DNA (cfDNA) in blood dates back to 1948 when Mandel and Métais observed circulating DNA and RNA in human plasma [24]. The implications of this discovery remained obscure for decades, until Leon et al. reported increased concentrations of cfDNA in the circulation of cancer patients in 1977 [25]. After another decade Stroun et al. [26] provided conclusive evidence of neoplastic ctDNA in the serum of cancer patients. The definitive link came in 1994, when Sorenson et al. [27] detected mutated *KRAS* oncogene sequences in plasma cfDNA by allele-specific polymerase chain reaction (PCR) and convincingly linked the mutant DNA fragments to the original patient tumor. These investigations, over the course of nearly half a century, opened a door to the potential utility of analyses of ctDNA in blood for cancer diagnosis, prognosis, and treatment.

The process by which tumor DNA enters into circulation, consequently, has been of interest, and studies [28–30] have revealed multiple mechanisms (Figure 1A). Migrating tumor cells can enter the bloodstream directly as CTCs (not a topic for this review, see [31]) and may contribute as a source of ctDNA. Actively growing tumors also experience periods of heightened apoptosis or necrosis, processes demonstrated to release DNA into circulation [32]. Tumor cells closely interact with vascular cells and are shown to shed DNA into circulation. These ctDNAs maintain tumor-specific genetic and epigenetic aberrations; including point mutations in tumor suppressors and oncogenes [22, 33], copy number variants [34, 35], DNA methylation patterns, and chromosomal rearrangements [36]. The advantages of noninvasive blood collection over surgery, or even needle biopsy, to identify the genetic and molecular defects reflective of the tumor mass(es) have produced tremendous interest in liquid biopsy. It is envisioned that blood testing could be applied to cancer screening, early detection, evaluation of tumor heterogeneity, observation of dynamic changes, identification of genetic/epigenetic alterations for targeted therapy, and assessment of drug resistance (Figure 1B). Utilization of ctDNA, through implementation of liquid biopsy can provide a new era of comprehensive genomic profiling during the full course of disease, from initial diagnosis through treatment and progression.

Recent growth in literature attests to the value of ctDNA in cancer diagnosis, prognosis, and monitoring of disease progression and therapy response (Figure 2 and Table 1). In 2005, methylation in ctDNA was shown useful for early detection [37] and much later as a diagnostic tool for monitoring cancer dynamics [38]. In 2006, Kimura et al. [39] demonstrated the role of *EGFR* mutations in predicting response to treatment with molecularly targeted inhibitors; a similar line of study was reported by Kuang et al. [40]. In 2008, ctDNA dynamics measured in patients undergoing treatment for colorectal cancer (CRC) reflected tumor responses and progression, and ctDNA detection after surgery indicated residual disease [41]. In 2013, Murtaza et al. [42] described utility of exome sequencing of cfDNA from serial plasma samples to study clonal evolution and to track ctDNA dynamics in high-burden disease. Additional examples follow.

Detection and quantification of ctDNA: technological development

Clinical applications for liquid biopsy are largely driven by technology development from genomics research. Applications include real-time PCR (qPCR) [43, 44]; the Scorpion Amplification-Refractory Mutation System [45]; Beads, Emulsion, Amplification and Magnetics [46]; digital PCR

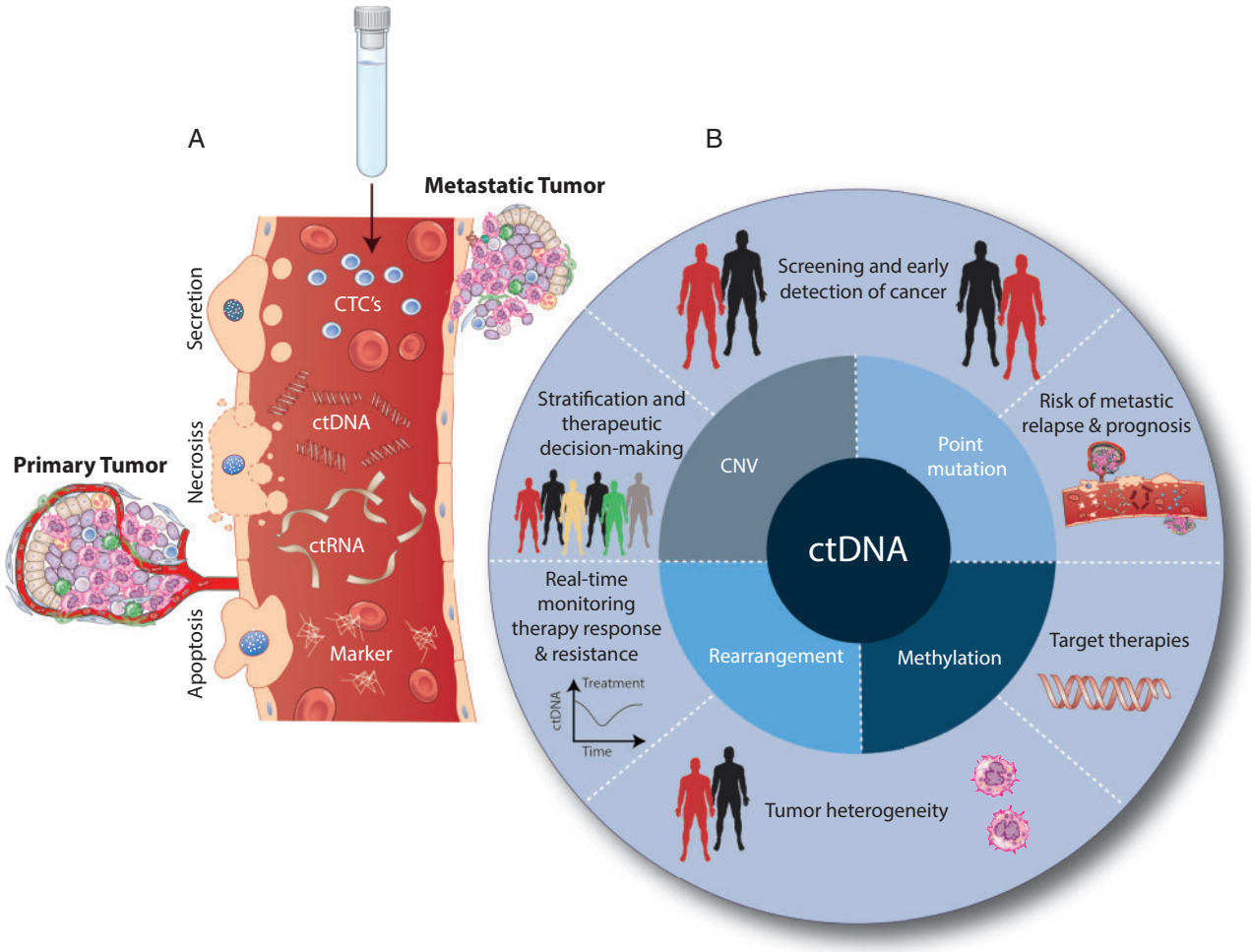

Figure 1. Biological characteristics and clinical applications of circulating tumor DNA (ctDNA). (A) Circulating tumor cells (CTCs), ctDNA, circulating tumor RNA (ctRNA), and circulating tumor protein as complementary blood-based biomarkers. Tumor cells release ctDNA into the bloodstream through apoptosis, necrosis, and secretion. A subset of aggressive ctDNA enters the bloodstream from the primary tumor or metastatic lesions. (B) Central circle illustrates ctDNA as having multiple classes of genetic and epigenetic alterations. Outer circle presents the potential clinical benefits of ctDNA monitoring in cancer management. CNV, Copy Number Variation.

(dPCR) [47, 48]; and NGS [49, 50]. Being most broadly applicable, dPCR and NGS are briefly described.

dPCR to identify targetable mutations and track targeted therapy responses

The dPCR approach expands quantification of gene expression beyond traditional qPCR by directly quantifying the exact number of target molecules. In this manner, dPCR allows detection of a single copy of mutated ctDNA, even in complex mixtures, rendering it highly sensitive [51]. Importantly, dPCR has a relatively simple workflow facilitating implementation in the clinical laboratory.

NGS to screen and detect disease burden

NGS refers to high-throughput genome sequencing using one of several available platforms. Through parallel sequencing of millions of DNA templates, NGS reveals a large portion of the genome. The richness of NGS is realized in the diversity of genomic input material (whole-genome, exome, de novo, targeted, RNA, ChIP, methylation, etc.) and analysis pipelines. Currently, multiple applications are used in oncology, such as targeted sequencing (gene panels) and whole-exome or whole-genome sequencing [14, 21, 50, 52, 53].

Each analysis method has its own diagnostic niche. dPCR is rapid, relatively inexpensive, and allows quantitation of mutant alleles, particularly at very low concentrations. A significant constraint, however, is required a priori knowledge of specific mutations for analysis, with limited detection of rearrangements, and analysis of many mutations presenting a challenge. NGS allows multiplex analysis of thousands of genomic positions and readily detects rearrangements and copy number variation. There is a growing consensus that NGS will be the test of the future but the current downside is high cost of the deep sequencing necessary for high sensitivity. With technological demand leading to innovation, however, the detection capabilities should logically lead toward cost-effective gene coverage. Of note, GRAIL—a new company with a mission to facilitate early detection of cancer—is pioneering in this direction by development of higher coverage and deeper sequencing depth that is poised to significantly

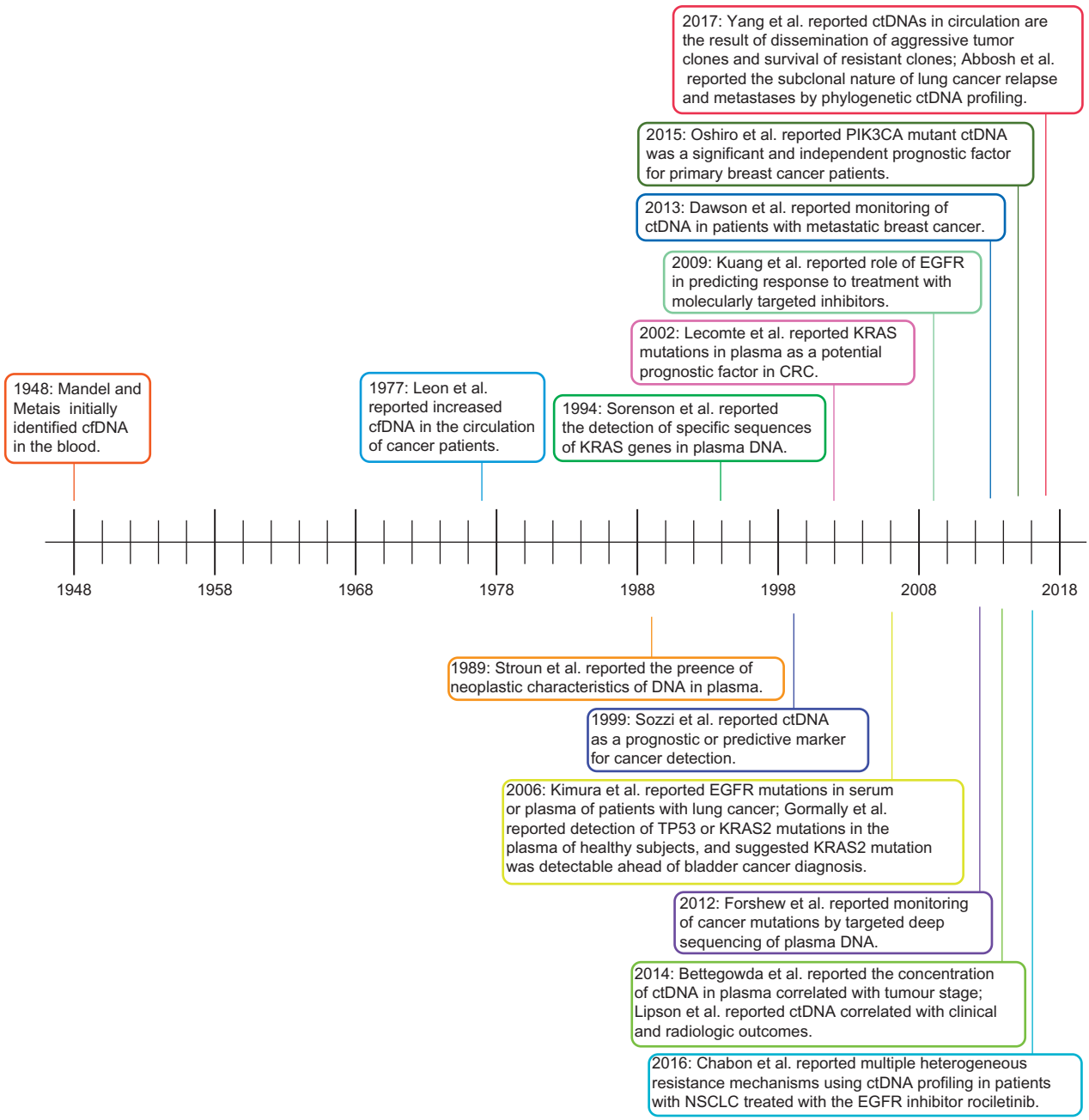

Figure 2. ctDNA discovery and concomitant developments in cancer: a timeline of genomics advances impacting cancer research over the past 70 years. Landmark discoveries are indicated on the timeline; the numbers of which have increased dramatically in the past 10 years owing in large part to next-generation sequencing capabilities.

circumvent these limitations [54, 55]. Our recent study [22] showed that even a single mutation in blood from cancer patients was diagnostic of decreased survival. Moreover, mutation rates in plasma were cancer stage-independent, supporting wide-ranging early diagnostic relevancy of a sensitive NGS-based approach. Newman et al. introduced an economical, ultrasensitive modification for quantifying ctDNA: Cancer Personalized Profiling by deep Sequencing (CAPP-Seq) to expand ctDNA detection for broad clinical applicability [56]. The use of CAPP-Seq in non-small-cell lung cancer (NSCLC) revealed emergence of drug-specific mutational patterns associated with resistance to EGFR-targeted therapies [57]. In a majority of patients analyzed, post-treatment residual disease detected at the molecular level by CAPP-Seq preceded cancer progression detected by radiographic measures by a median of 5.2 months [58]. MSKCC researchers using the Grail platform for NGS, reported detection of mutations in circulation in 89% of 151 metastatic cancer patients using ultra broad coverage (508 genes) and ultra-deep sequencing (60 000×) [59]. Accumulating evidence suggests that ctDNA detection techniques are quantitative and that changes in ctDNA levels during chemotherapy are associated with tumor response or progression in several tumor types [41, 60–62]. These studies

Table 1. Trends in ctDNA literature by cancer type

Cancer types	1989–1999	2000–2004	2005–2009	2010–2014	2015–
NSCLC	1 [104]		6 [39, 40, 105–108]	22 [49, 50, 56, 76, 79, 82, 84, 89, 90, 109–121]	21 [10, 14, 16, 47, 52, 57, 64, 83, 85, 122–133]
SCLC					1 [134]
Lung cancer			1 [78]		1 [135]
CRC		1 [77]	2 [41, 136]	12 [48, 98, 137–146]	9 [15, 62, 87, 147–152]
Colon cancer					1 [73]
Breast cancer				4 [66, 69, 153, 154]	7 [8, 61, 67, 99, 155–157]
Ovarian cancer			1 [158]	1 [159]	2 [160, 161]
HCC				2 [34, 162]	2 [163, 164]
Brain tumor					1 [165]
Pancreatic adenocarcinoma	2 [27, 166]				1
Bladder cancer					1 [167]
Prostate cancer				1 [35]	2 [53, 168]
Renal cell carcinoma				1 [1]	
Melanoma				1 [169]	5 [88, 170–173]
Lymphoma					1 [174]
Nasopharyngeal carcinoma		1 [175]		1 [176]	
Various type	1 [26]	1 [32]	2 [177, 178]	4 [36, 42, 71, 179]	9 [21, 22, 97, 180–185]

Note: The number of publications followed by the references in the bracket are shown in the table.

begin to demonstrate the clinical utility of ctDNA detection at all stages of disease.

New insight into cancer hallmarks through ctDNA

Our current understanding of cancer is derived primarily from investigation by cancer types and their tissues of origin. The systems for staging and for designing cancer therapeutics also rely on this information. The unprecedented ability to interrogate cancer as a systemic disease by examining ctDNA has led to a number of new perspectives.

Tumor heterogeneity and clonal evolution

Cancer is a heterogeneous disease with respect to the molecular mechanisms underlying its development. Solid tumors also exhibit temporal heterogeneity, evolving spontaneously over time and shaped by responses to selection-pressures, such as the immune system and treatment. Almost all cancers treated with anticancer agents have the capacity for resistance as a function of tumor heterogeneity, clonal evolution, and selection [63].

Jamal-Hanjani et al. [64] prospectively investigated intratumor heterogeneity in relation to measures of clinical outcome, clonal nature of driver events, and evolutionary processes in early-stage NSCLC. They reported that driver mutations of *EGFR*, *MET*, *BRAF*, and *TP53* were almost always clonal. In addition, heterogeneous driver alterations occurred later in evolution in more than 75% of tumors, commonly in *PIK3CA* and *NF1* and genes involved in chromatin modification and DNA damage response and repair. Gerlinger et al. [1] observed intratumor heterogeneity in renal carcinoma, detecting a minority of the total genetic burden in any one biopsy. Of note, among compartmentalized sites within the same tumor, they report convergent evolution events among several tumor suppressor genes leading to loss of function. This complex level of mutational heterogeneity highlights consequences for patients if treatment decisions depend on results from an initial, prototypical tumor biopsy. Consequently, relevant mutations might be overlooked.

Tumor subclones may arise during disease progression, altering the proportion and pattern of specific aberrations between the primary tumor and metastases [65]. The analysis of ctDNA addresses this issue, because ctDNA released from multiple tumor regions may reflect both intratumoral heterogeneity [66] and clonal evolution [16]. Evidence demonstrates that ctDNA from plasma reveals this clonal tumor hierarchy in cancer. Murtaza et al. [67] extensively analyzed sequential tumor tissue biopsies and plasma ctDNA samples in a ER+/HER2+ breast cancer patient over 3 years, including multiple metastatic deposits, and determined that ctDNA reflected the dynamics of clonal evolution over disease progression. CAPP-seq screening of ctDNA by Chabon et al. [57] identified multiple heterogeneous resistance mechanisms after EGFR inhibitor treatment in patients with NSCLC. They described a novel *EGFR* L798I mutation and found that *EGFR*-C797S, which arises in ~33% of patients after osimertinib treatment, occurred in <3% after rociletinib. Increased *MET* copy number was the most frequent rociletinib resistance mechanism and patients with multiple preexisting mechanisms (*EGFR*-T790M and *MET*) had inferior responses. Interestingly, Abbosh et al. [16] demonstrated the subclonal nature of lung cancer relapse and metastases by a new tumor-specific phylogenetic approach using ctDNA profiling. They showed that mean plasma variant allele frequency of clonal SNVs was higher than that of subclonal SNVs, supporting use of clonal alterations as a more sensitive method of ctDNA detection than subclonal alterations. They also demonstrate the feasibility of using ctDNA platforms to guide drug development, identify residual

disease, and target emerging subclones before clinical recurrence in NSCLC. Yang et al. [22] demonstrates a key goal of tumor heterogeneity investigations is to identify clinically aggressive or therapy-resistant clones. Analysis of DNA mutations in lung tumor tissue compared with liquid biopsy from the same patients revealed that mutations in *TP53*, *EGFR*, *BRAF*, *CTNNB1*, *ARID1A*, *ERBB2*, and PDGFRA present in minor tumor clones were detectable in plasma. This study points to ctDNA in circulation as a meaningful indication of dissemination of aggressive tumor clones and survival of resistant clones. Thus, ctDNA analyses have provided direct evidence of spatial and temporal intratumor heterogeneity and show that the range of subclonal heterogeneity is variable among cancers.

ctDNA improves early detection of metastasis

The metastatic process is a complex evolutionary progression of cellular events whereby malignant cells from the primary tumor become migratory and move into the circulation, either directly via a blood vessel or indirectly via a lymphatic vessel, to finally inhabit distant sites as metastases [5]. Since tumor biopsy from one site may not completely reveal the genomic landscape of a patient's disease burden, blood ctDNA analysis to characterize cancer subclones would help guide treatment decisions. Deryugina et al. [68] illustrated that metastasis can occur throughout tumor progression, even early stage. Dawson et al. [69] compared radiographic imaging of tumor progression to assays detecting ctDNA, cancer antigen 15-3, and CTCs in 30 women receiving systemic therapy for metastatic breast cancer. Of these biomarkers, ctDNA levels showed the greatest detection sensitivity, dynamic range and correlation to disease burden, importantly providing an average 5-month advantage over CT imaging in detecting disease progression in 53% of patients. The utility of ctDNA detection in late stage monitoring extends applicability to early detection since ctDNA alterations are identified in blood from patients with early stage disease [70, 71]. Higher ctDNA levels in early stage cancers may predict more rapid progression to late stage disease. Notably, Naxerova et al. [72] recently illustrated that lymph node metastases may not always be the source of cancer's spread to other organs; in 65% of cases lymphatic and distant metastases in CRC arose from independent subclones from the primary tumor. An ultrasensitive approach recently developed at Johns Hopkins demonstrated that ctDNA detects early-stage tumors and that ctDNA levels are associated with disease recurrence and decreased overall survival [23]. Tie et al. also used NGS-based assays to evaluate ctDNA in 1046 plasma samples from a prospective cohort of 230 patients with resected stage II colon cancer; they demonstrated ctDNA detection after resection provides direct evidence of residual disease and identifies patients at very high risk of recurrence [73].

Early detection of ctDNA may identify driver mutations and metastatic markers during tumor progression. Although patients with early stage or minimal residual disease usually have lower levels of ctDNA, deep sequencing may detect specific alterations to allow therapeutic intervention with the goal of preventing metastatic progression [73]. Hence, ctDNA could offer comprehensive insight into a patient's disease, even at an early stage, long before clinical manifestations of disease progression [74].

Prognostic and predictive implications

ctDNA detection may more accurately estimate patient prognosis. Previous studies have shown that plasma-based testing and detection of molecular heterogeneity can predict patient outcome [75, 76]. In a retrospective study of patients with stages I–III CRC, detection of ctDNA implied a higher risk of recurrence or shorter overall survival in patients treated with surgery, chemotherapy, radiotherapy, or targeted therapy [77]. High levels of *KRAS* mutated plasma DNA have been reported as an indicator of poor outcome in lung cancer patients [78, 79]. Sirera et al. [80] found that high pretreatment levels of circulating DNA acted as an independent prognostic marker for shorter survival. Moreover, *KRAS* status in plasma ctDNA was associated with poor tumor response to *EGFR* tyrosine kinase inhibitors (TKIs) in NSCLC patients and served as a predictive marker for selecting appropriate treatments [78, 81–84]. In a retrospective analysis [85], patients with NSCLC who were positive for *EGFR*-T790M in plasma showed outcomes with osimertinib equivalent to patients classified as positive by a tissue-based assay, supporting plasma analysis to avoid invasive tumor biopsies in such patients. A systematic review including 23 studies reported that the presence of ctDNA in blood is associated with worse survival in patients with solid tumors [86]. Taken together, these studies propose that ctDNA measures provide insight for patient prognosis that could further inform clinical decision-making.

Monitoring of treatment responses

Detection of molecular aberrations in ctDNA provides a powerful tool to monitor response to therapy and emergence of secondary mutations associated with therapy resistance [22, 61, 76, 87, 88]. Given the dynamic nature of cancer, ctDNA investigation at multiple time points during cancer treatment and progression may provide crucial information for patient management. Studies have reported that first-line treatment of patients harboring *EGFR* activating mutations with EGFR TKIs gefitinib, erlotinib, or afatinib results in superior overall response rates, progression-free survival and quality of life compared with chemotherapy [40, 83, 84, 89, 90]. Detection of *EGFR*-T790M mutation at follow-up facilitates treatment with the third generation EGFR TKI Osimertinib [59]. Thress et al. [10] reported, however, that serial ctDNA monitoring of lung cancer patients treated with Osimertinib revealed a diversity of therapy resistance mechanisms. Cabel et al. [91] demonstrated that quantitative ctDNA monitoring was a valuable tool to assess tumor response in five metastatic melanoma patients treated with anti-PD1 drugs [91]. Furthermore, longitudinal assessment of ctDNA in metastatic melanoma patients who were treated with PD1 inhibitors was an accurate predictor of tumor response and overall survival [92]. Vidal et al. determined baseline *RAS* at diagnosis and monitored of the emergence of *RAS* mutations as a mechanism of resistance to anti-EGFR therapy [93]. In advanced prostate cancer, ctDNA can detect aberrations in the androgen receptor and may help to predict for response or resistance to androgen directed therapies [94]. Thus, in such an aggressive disease targeted therapy success is entwined with vigilant monitoring of treatment response. Critically, ctDNA genomic alterations over disease progression provide real-time therapeutic guidance, predict prognosis and assess for therapy resistance ahead of imaging studies.

ctDNA early detection for staging and auxiliary diagnostic screening

The amount of ctDNA detected in blood is correlated with cancer stage and tumor aggressiveness [95]. Comparing late to early

stage disease, ctDNA was detected in 100% of patients with stages II–IV NSCLC and in 50% of patients with stage I NSCLC [96]. ctDNA detection shows some variation among tumor types. A high proportion of patients with advanced primary pancreatic, ovarian, colorectal, breast, bladder, esophageal, melanoma, and hepatocellular carcinoma have measurable ctDNA while detection falls under 50% for patients with brain, renal, prostate, and thyroid cancers [71], though this will likely vary with the technology used for ctDNA detection. The same group reports detecting ctDNA from about 50% or more of patients with localized disease (colorectal, esophageal, pancreatic, and breast adenocarcinoma). Moreover, a study with 95% of patients having advanced or metastatic disease reported that 58% of patients had at least one detectable alteration, which increased to 65% when glioblastoma was excluded [97]. In their comprehensive quantitative analysis, Bettegowda et al. [71] reported varying levels of ctDNA across patients with distinct cancer types and provided expected ranges of ctDNA levels across the stages of disease.

Quantifying ctDNA levels enables earlier response assessment than standard radiographic approaches. Misale et al. [98] reported serial ctDNA analysis identified *KRAS*-mutant alleles in the plasma of cetuximab-treated patients 10 months before disease relapse was identified by imaging. They suggest that ctDNA could supplement standard screening or restaging approaches for cancers—such as mammography for breast, low dose spiral CT for lung, colonoscopy for CRC, and PSA for prostate—potentially increasing diagnostic sensitivity and specificity. In a study designed to monitor treatment response, Garcia-Murillas et al. [99] used NGS of tumor biopsies in patients with early stage breast cancer to identify patient-tumor-specific mutations and developed personalized dPCR assays to detect and track ctDNA in 55 patients receiving neoadjuvant chemotherapy followed by surgery: detection of ctDNA after surgery or during serial sampling was a significant predictor of early relapse. Furthermore, ctDNA provided a median lead time of 7.9 months before discovery of clinical relapse. Similarly, Olsson et al. [61] used ctDNA for early detection of metastasis in women who presented with early stage, nonmetastatic breast cancer and received no neoadjuvant therapy; reporting 13 of 14 patients with eventual clinical recurrence showed positive ctDNA levels postoperatively, whereas patients with long-term disease-free survival had no detectable ctDNA. Critically, ctDNA molecular detection of occult metastasis preceded the clinical diagnosis in 12 of 14 patients with an average lead time of 11 months.

These studies indicate that ctDNA detected in the blood of early stage patients is a robust and independent marker of disease progression, which may significantly enhance the current prognostic assessment. Doctors have already begun to order ctDNA and NGS tests as part of precision oncology programs. Because of the noninvasive nature, implementation of ctDNA testing is straightforward but utility must develop from a network encompassing research knowledge, product/test development and clinical validation.

Proposing a modified cancer staging system for solid tumors: TNMB

The Tumor, Node, and Metastasis staging system was devised by Pierre Denoix during 1943–1952 [100] to classify malignant tumors with a goal of standardizing treatment regimens and survival expectations by providing a uniform guide to describe the anatomical extent of disease. Since introduction, the TNM system has gained wide international acceptance for staging solid tumors. Applying TNM taxonomy criterion, cancers are staged into one of four groupings (I, II, III, and IV) for a diagnosis that provides a prediction of treatment course and disease prognosis. Stage IV, exclusively indicating distant metastases, represents late stage disease and generally poor prognosis, thus imploring any mechanism to better identify systemic disease as being highly meaningful. While prognostic biomarker research in cancer has great potential to drive personalized medicine, the complexity of implementation is equally great. A logical first step, quantifying ctDNA levels in blood, offers a practical solution to the quandary.

In light of new genomic technologies that improve assessment of risk for cancer progression and/or metastatic disease, the time has come to consider amending the TNM system [101]. We propose a modified staging system for future development—'TNMB'. Figure 3 shows how TNMB (B represents blood) complements TNM staging by adding a liquid biopsy 'B' to capture prognostic and therapeutic implications gained from ctDNA evaluation. Paralleling the 'M' category, initial categorization may be defined as the absence ('B0') or presence ('B1') of detectable ctDNA. Although in need of standardized criteria, B staging should apply to most cancers independent of site. As literature accumulates, classification could be refined to include ctDNA quantification data with clinically meaningful cut-offs by site [71, 102]. Future incorporation of data such as mutational burden, actionable mutations, and metastasis-related mutations will further enhance the clinical impact of the 'B' designation. Foundation Medicine has recently put forward ctDNA mutation load as an end point for their liquid biopsy test because of demonstrated clinical utility [63]. Prospective studies to compare TNM staging to TNMB staging on their accuracy in predicting cancer recurrence and patient survival are critically needed and it is likely that the growing body of literature on ctDNA will help define clinically meaningful categories within the 'B' designation for each tumor type.

Discussion

Conclusions

Beyond simply reproducing information from tissue biopsies, noninvasive ctDNA analysis offers a comprehensive and integrated view of the systemic evolution of cancer. ctDNA has been shown to be a relevant blood-based biomarker useful as a complementary method for cancer screening and diagnostic tool. By providing material for mutational analysis in the clinical setting, ctDNA facilitates highly sensitive monitoring for the acquisition of treatment resistance. Results of ctDNA detection have been successfully used to guide targeted therapies aiming at key driver events for metastasis. Consequently, we propose that development of a TNMB staging system to include blood ctDNA information to enhance the current TNM cancer staging system. This new component will stimulate further development of specific and sensitive cancer detection technologies for blood biomarkers in the rapidly evolving field of precision oncology. We envision

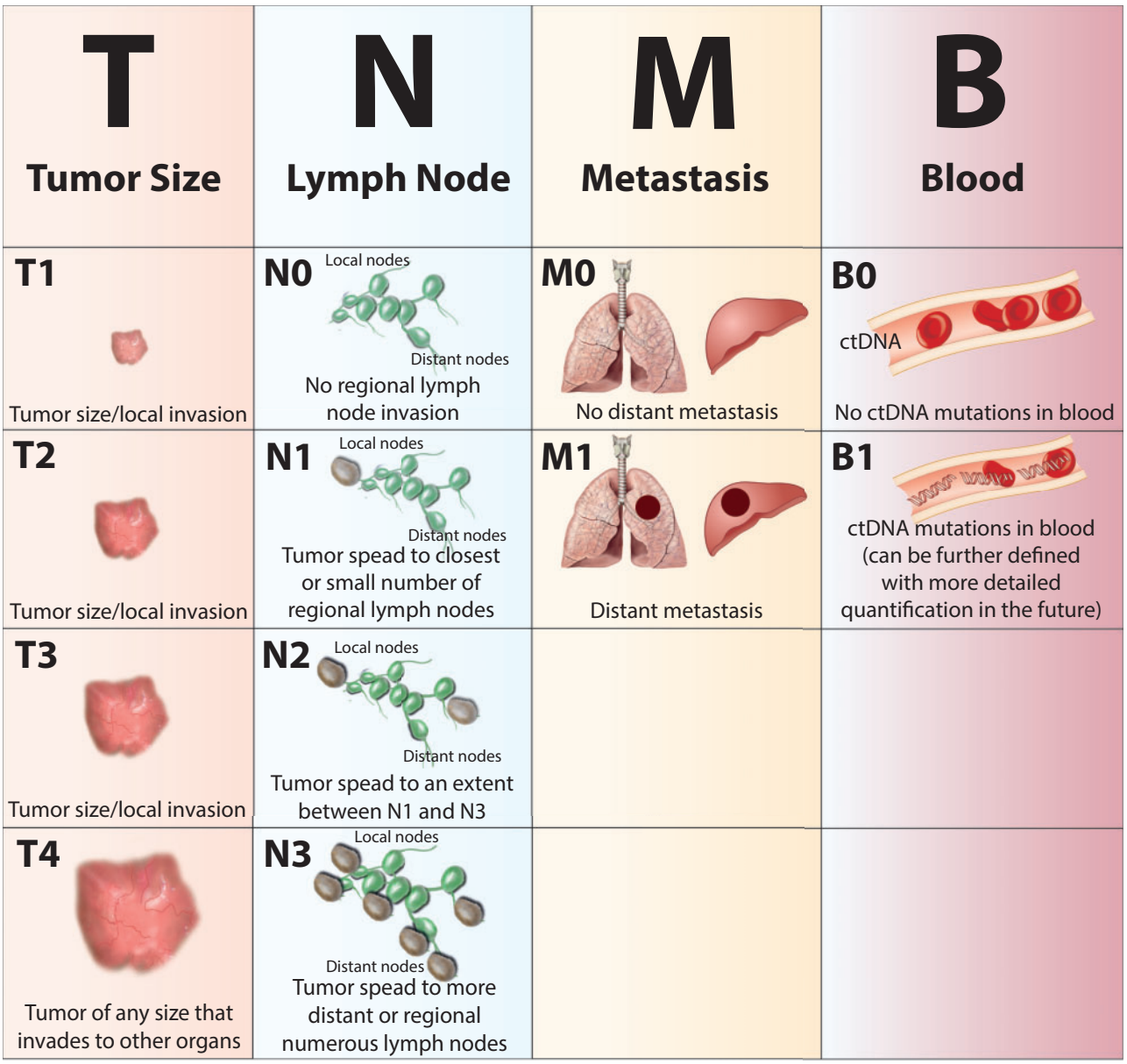

Figure 3. Generalized overview showing the proposed ctDNA incorporation into the hallmark cancer staging system as TNMB. Each categorical column indicates the anatomical valuation criteria by row for advancing cancer stage, thus reflecting tumor progression. Advancing genomics technologies promise to reveal this previously hidden anatomical indicator—ctDNA— to more accurately stage and treat cancers. Information for TNM staging in this figure derived from the *TNM Classification of Malignant Tumours* by UICC (7th Edition) [103].

an iterative process is needed where clinical data from population-based cohorts are compiled to evaluate survival associated with staging groups. To initiate use within the TNMB system, large data registries are needed to start capturing 'B' so that the next iteration of staging revisions can incorporate the important prognostic information from this variable with the hope to improve outcomes for all cancer patients.

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