Editorial

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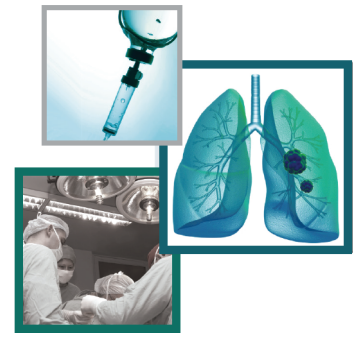
Lung Cancer Management



Optimizing molecular residual disease detection using liquid biopsy postoperatively in early stage lung cancer

Anna L McGuire*[,1], Curtis B Hughesman[2], Melissa K McConechy[3], Barb Melosky[4,5], Stephen Lam[4,6], Renelle Myers[4,6], John Yee[1], Ernest Tang[1] & Stephen Yip[2,7]

[1]Department of Surgery, Division of Thoracic Surgery, Vancouver General Hospital, University of British Columbia, Vancouver V5Z1M9, BC, Canada
[2]Department of Pathology and Laboratory Medicine, BC Cancer, Cancer Genetics & Genomics Laboratory, Vancouver, BC, Canada
[3]Contextual Genomics Inc., Vancouver, BC, Canada
[4]Department of Medical Oncology, BC Cancer Agency, Vancouver, British Columbia, Vancouver, BC, Canada
[5]Medical Oncology, University of British Columbia, Vancouver, BC, Canada
[6]Department of Medicine, Divisions of Respiratory Medicine, University of British Columbia, Vancouver, BC, Canada
[7]Department of Pathology & Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada
*Author for correspondence: anna.mcguire@vch.ca

"Stage I/II NSCLC surgical patients with MRD undergoing liquid biopsy postoperatively would be the ideal target population for assessment of the impact of adjuvant targeted systemic therapy on disease-free survival."

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Lung cancer remains the number one cause of cancer death globally [1,2]. Unfortunately, in an unscreened population, the majority of patients diagnosed with lung cancer (80%) present in stage III/IV [3,4]. Patients with the best prospect for cure include those with early stage (I/II) NSCLC undergoing curative intent surgical therapy. These early-stage patients have no clinical or radiological signs of overt cancer. Despite receiving curative intent treatment, many still experience recurrent disease and poor long-term survival [5]. This may be due to occult molecular residual disease (MRD) that is undetectable with current standard technology, yet is an important source for future cancer recurrence [6].

Existing postoperative detection methods are limited to microscopic residual disease assessment of the surgical specimen margins (traditional histopathology), and gross disease detection with imaging (positron emission tomography and CT scans) [7]. Liquid biopsy for ctDNA in patient blood has been identified as an attractive, noninvasive modality for detection of MRD after curative intent local therapy [8–12].

Stage I/II NSCLC surgical patients with MRD undergoing liquid biopsy postoperatively would be the ideal target population for assessment of the impact of adjuvant targeted systemic therapy on disease-free survival. In the context of a clinical trial, blood-based stratification of the study population by presence of genetic alterations for targeted systemic therapy selection is the ideal avenue for introduction of this technology into postoperative clinical practice [13].

Despite the attractive potential, several limitations must be overcome in order to offer this testing reliably for the postoperative MRD clinical setting. Here, we discuss key theoretical and technical principles relating to the limit of detection, and strategies to increase the probability of ctDNA detection in cell free DNA for analysis using highly sensitive next generation sequencing (NGS). The following discussion assumes optimized laboratory plasma isolation and DNA extraction techniques, as these will inherently impact assay sensitivity with respect to both cell free DNA yield and quality [14].

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Most healthy individuals rarely have more than 10 ng of cell free DNA per ml plasma, most of which originates from hemopoietic cells [14–16]. Although much remains to be learned about diurnal variation and clearance mechanisms of cell free DNA [17–20], various physiologic stress events have been shown to influence its release at the cellular level from noncancerous host cells. Such cell death inducing events include: intense exercise [21,22], autoimmune disease [23], stroke [24,25], myocardial infarction [26], trauma [27] and sepsis [28]. The fraction of cell free DNA in patient blood that arises from the tumor itself is referred to as ctDNA. Fragmented tumor-specific DNA can be shed into blood passively via apoptosis, necrosis, phagocytosis or actively with the secretion of microvesicle exosomes [13,29–32]. The ctDNA levels in blood are generally low, and have been shown to be directly proportional to tumor burden and clinical stage in NSCLC and other solid tumors [33–35]. Cell free DNA has a short half-life in the circulation, with a range of 16–150 min reported [17,20]. As such, blood-based detection of the ctDNA molecular portion has potential to be a real time measure of tumor burden.

Several targeted plasma liquid biopsy ctDNA platforms have been previously reported for use in NSCLC [12,36–41]. Such highly sensitive targeted NGS panel assays routinely report the ability to detect a level of variant allele frequency (VAF) in plasma, which often assume that enough cell free DNA is available to identify these extremely low frequency variants.

In the setting of early stage NSCLC cancer however, it is valuable to conceptualize how VAF depends on both total cell free DNA input for testing, and number of ctDNA molecules present in patient blood for sampling [14]. For instance, the quantity of ctDNA in the cell free DNA fraction in patients with stage I tumors has been measured to be approximately tenfold lower than that in patients with stage IV disease [12,13]. In the postoperative MRD setting, based on the inherent decreased quantities of tumor DNA shedding from early stage NSCLC, the importance of detecting alterations present at a VAF of $<0.1\%$ has been established [8,9,12,33]. The probability of detecting a certain number of ctDNA molecules at a low volume in blood follows the Poisson distribution, where not all blood samples will be 'positive' even with a method that can detect individual ctDNA molecules [42]. Johansson *et al.* have demonstrated that if it is assumed that one ctDNA molecule is required for genetic alteration detection, in order to fully maximize the utility of NGS approaches ≥ 3.6 ng total input cell free DNA is necessary [14]. Practically this implies that workflow improvements for cell free DNA yield may improve technical sensitivity.

So, how can we as clinician scientists practically increase the probability of ctDNA molecular capture postoperatively? Analyzing larger patient blood volumes to increase the probability of ctDNA molecule detection is one potential avenue to increase limited input DNA [13]. An additional method is to use existing knowledge of the genetic alterations present in the lung tumor tissue to improve analytical sensitivity. Use of matching cancer panels for targeted genetic alteration detection in both lung tumor tissue and plasma may increase the lower limit of ctDNA detection [33,36,39]. Digital PCR on postoperative plasma may then be conducted as needed for known alterations identified in tumor tissue that may be present at low frequency in plasma for quality assurance in optimization of ultrasensitive multiplex cancer panels [13].

Matching reference genetic alterations in resected lung tumor tissue with blood plasma sequencing panels also mitigates the problem of false-positive results from clonal hematopoiesis of indeterminate potential (CHIP). This phenomenon increases with age and may give rise to genetic variants in the blood unrelated to the lung tumor of interest that confound ctDNA detection [43]. CHIP is especially problematic is the setting of MRD detection where the VAF is low [44]. However, CHIP can also be accounted by sequencing matched plasma buffy coat samples to similar depth as ctDNA [43–45]. The probability of ctDNA detection has also been shown to increase with use of multiplex assays, analyzing simultaneously for many independent genetic alterations [14].

Additional advanced NGS methods to increase sensitivity with limited input DNA include both use of unique molecular identifiers either exogenously attached or endogenously inferred from sequence context, and sophisticated error correction bioinformatics analytical approaches [14,37,46]. These advanced methods together help address the technical limitations for detection of low frequency genetic alterations by NGS due to artefactual errors that accumulate during library preparation, target enrichment and sequencing. Artefactual errors are problematic as they lead to false-positive mutations and can mask detection of true biological variants [39,46]. Unique molecular identifiers are short sequences that incorporate a unique barcode onto each molecule in a given tumor DNA sample library. They have been shown to reduce the frequency of false-positive variant calls and increase sensitivity of true low frequency genetic variant detection [37]. The value of incorporating barcodes onto original ctDNA fragments is that the true variant alleles present in the original plasma ctDNA sample can be distinguished from errors introduced during library preparation, target enrichment or sequencing. Any such errors identified can then be removed by bioinformatics analytical approaches.

As outlined in our discussion above, precise methods of ctDNA detection in early stage surgical NSCLC have the potential to transform postoperative clinical practice via noninvasive MRD detection. The identification of tumor-specific genetic alterations using tissue or blood-based NGS tests are helping to drive personalized treatment and remains an important ongoing area of study. There are still no clinically standard NGS assays to identify MRD postoperatively. Ongoing pilot studies in this setting are essential in efforts to decrease the global burden of disease and mortality due to lung cancer.

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