

## ctDNA to detect minimal residual disease in pancreatic cancer: moving into clinical trials

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Incidence rates of pancreatic ductal adenocarcinoma (PDAC) continue to increase every year and PDAC has recently become the forth-leading cause of cancer-related death in Western countries <sup>1,2</sup>. Surgery plus neoadjuvant or adjuvant therapy is currently the only strategy to cure PDAC patients but less than 15% will be diagnosed in the localized setting and, even in this subset of patients, 5-year overall survival (OS) rates remain below 30%. A survival improvement has been shown with adjuvant mFOLFIRINOX or gemcitabine plus capecitabine, compared to the standard treatment with gemcitabine alone <sup>3,4</sup>. However, combination regimens significantly increase toxicity and only selected patients will be eligible to receive these schemes <sup>5,6</sup>. Despite improvement in treatment outcomes, we are still facing high rates of recurrence after adjuvant therapy, and validation of accurate predictive biomarkers are urgently needed to better guide treatment intensity and provide prognostic information to patients and clinicians.

Sequencing of pancreatic cancers DNA has identified four driver genes that are recurrently somatically mutated: *KRAS*, *TP53*, *SMAD4* and *CDKN2A* <sup>7,8</sup>. Particularly, *KRAS* exon 12 mutations are found in more than 90% of PDAC <sup>9</sup>. These tumor-specific DNA mutations can be detected in the cell-free component of peripheral blood [circulating tumor DNA (ctDNA)] in most patients with metastatic PDAC <sup>10,11</sup> allowing for a noninvasive characterization of the tumoral molecular profile.

In the current issue, Lee and colleagues <sup>12</sup> evaluate the prognostic role of *KRAS* ctDNA in a prospective cohort of patients with resectable PDAC. Using a PCR-based SafeSeq assay, the preoperative sensitivity for ctDNA detection was 62% (23 ctDNA positive out of 37 *KRAS* mutated tumors in tissue). Positive *KRAS* ctDNA identified a subset of patients with poorer outcome compared to patients with negative *KRAS* ctDNA [Hazard Ratio (HR) for recurrence free survival (RFS) of 4.1; p=0.002 and HR for OS 4.1; p=0.015], as shown in other tumor types <sup>13,14</sup>.

Despite the important technological advances in ctDNA analysis in recent years, preoperative ctDNA detection in localized solid tumors still ranges from 50 to 70%<sup>10,15-18</sup>, mostly due to the intrinsic tumor biology rather than a lack of technical sensitivity. Non-shedding tumors, stromagenic microenvironment, poorly vascularized tumors or natural barriers as the blood-brain barrier are some of the potential tumor-related reasons why ctDNA is not detected in a high proportion of patients. Moreover, the prospective trial design in early stage PDAC adds a layer of complexity to the current study: almost half of the patients had to be excluded because of inoperable tumors at the time of surgery, while one third of blood or tissue samples were insufficient or unavailable, leaving a total of 42 evaluable patients out of the 112 initially included. An accurate pre-surgical diagnosis as well as an effective protocol of sample collection, storage and analysis is of utmost importance to empower the use of liquid biopsies in trials and routine clinical practice.

Even more relevant are the results presented in ctDNA detection following surgery. All patients with positive ctDNA eventually relapsed (positive predictive value (PPV) 100%), whereas patients with negative ctDNA values were at lower risk for recurrence (negative predictive value (NPV) 54%). Interestingly, 10 out of 22 patients with negative postoperative ctDNA also relapsed, reflecting the aggressive behavior of pancreatic cancer, in which a high percentage of patients are eventually micrometastatic at the time of surgery.

From all the potential applications of liquid biopsies, the detection of somatic mutations in ctDNA as a surrogate of minimal residual disease (MRD) is currently one of the most explored and promising<sup>16,19,20</sup>. Nevertheless, this valuable source of information is diluted by much larger quantities of cell-free DNA (cfDNA) from noncancerous origins, and ctDNA usually represents only a small fraction of the total cfDNA in circulation. In this study, the authors used the Safe-Sequencing System to detect mutations in *KRAS* hotspots. This technology uses uniquely molecular barcodes attached to DNA fragments which increase the sensitivity to <0.1% and reduces false positive results due to errors in massively PCR sequencing<sup>21</sup>. Although the strategy of limiting ctDNA detection to a single gene may be useful in a tumor like PDAC where *KRAS* mutation rates exceed 90%, the biology of ctDNA release in the bloodstream and the fact that 10% of tumors do not harbor a *KRAS* mutation may underestimate the true circulating tumor burden. The use of a next generation gene panel including *KRAS*, *TP53*, *CDKN2A*, and *SMAD4* may increase the options for detection, but at a higher cost and decreased sensitivity. Another potential strategy to reduce the rates of false negative

ctDNA detection could be to collect multiple blood samples at different time-points (i.e., before, during or right after the adjuvant treatment).

The clinical implications of these results are relevant as it suggests that postoperative ctDNA analysis could be used as a biomarker of MRD, not only to identify patients at high risk of recurrence but to guide clinical trials to evaluate more intensive therapies for patients with detectable ctDNA after surgical resection. Following this aim, the authors designed the first prospective clinical trial that explores the efficacy of post-surgery ctDNA informed approach to guide adjuvant chemotherapy in localized PDAC (DYNAMIC-pancreas). Patients are randomized into two cohorts: cohort A (control) treated with standard of care chemotherapy and cohort B (ctDNA informed) in which patients are treated according to ctDNA results: “ctDNA-positive” patients receive an escalation chemotherapy strategy, whereas a de-escalated treatment strategy is offered to “ctDNA-negative” patients. Promising studies are also being conducted in other localized solid tumors that evaluate the clinical impact of ctDNA to guide adjuvant treatment strategy. Besides, ctDNA genotyping can take us a step further in the design of adjuvant clinical trials giving the opportunity to include the use of targeted therapies or even immunotherapy based on detected actionable mutations or MSI-H tumors (figure 1). Moreover, the novel concept of molecular metastatic disease referring to persistent ctDNA positive detection despite adjuvant systemic therapy without radiological relapse, is at least disruptive and opens a window of opportunity for the testing of metastatic regimens in patients with molecular metastatic disease.

Taken together, Lee *et al.* provide more evidence towards the promising clinical application of liquid biopsies to monitor MRD. However, dramatic clinical improvement and integration into routine practice will need strong evidence built upon prospective clinical trials that show an impact in survival outcomes. Results from ongoing ctDNA-guided prospective clinical trials in the adjuvant setting are eagerly awaited and will potentially change the way we treat cancer.

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**Figure 1: Design proposal for adjuvant clinical trials based on ctDNA analysis to detect minimal residual disease in different clinical scenarios**

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