

**NEWS**

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**CIRCULATING TUMOR DNA TO DETECT MINIMAL RESIDUAL DISEASE IN STAGE III COLORECTAL CANCER: MOVING TOWARDS CLINICAL IMPLEMENTATION**

It is currently well established that circulating tumor (ct) DNA is a powerful tool to detect the presence of minimal residual disease (MRD) after curative-intent surgery in stage III colon cancer patients, which strongly correlates with relapse and prognosis. This has been possible by incremental technological advances in the assessment of ctDNA which have led to the development of highly sensitive and highly specific ctDNA detection strategies. From a clinical point of view, the use of strategies to refine adjuvant chemotherapy (ACT) and surveillance after ACT in stage III colon cancer is a high unmet clinical need. Currently, all fit patients diagnosed with stage III colon cancer undergo ACT with a combination of a fluoropyrimidine and oxaliplatin which may have long-lasting and life-limiting drug-related toxicities, especially peripheral neuropathy. Afterwards, an intensive follow-up surveillance program including repeated computed tomography (CT) imaging is offered to all patients. It is estimated, however, that 70% of patients are cured with surgery alone and 70% of patients are cured after ACT. While prospective clinical trials evaluating the use of ctDNA to personalize adjuvant strategies and surveillance are ongoing, a deeper understanding of ctDNA dynamics in resected patients will help in the implementation of ctDNA in daily clinical practice.

In an inspiring publication in *Clinical Cancer Research*, Henriksen et al.<sup>1</sup> show in a prospective multicenter homogeneous cohort of 168 stage III colon cancer patients that the risk of relapse after surgery is seven times higher for ctDNA-positive compared with ctDNA-negative patients. Moreover, a 50-fold increase in relapse is shown for ctDNA-positive compared with ctDNA-negative patients directly after ACT.<sup>1</sup> This confirms previous publications by the same group and others on the high value of ctDNA to discriminate relapsing from non-relapsing patients in stage III colon cancer after surgery and after ACT.<sup>2,3</sup> The central finding and clinical added value of this elegant study is that, in an effort to increase the knowledge on the utility of ctDNA in the adjuvant setting, the authors analyze serial blood samples before, during and after ACT. By doing this, they first give new insights on a very relevant practical question: when is the best time for MRD assessment after surgery? Based on the fact that surgical trauma increases cell free (cf) DNA derived from patient's normal cells and dilutes ctDNA below levels of detection for at least 4 weeks, the authors show that ctDNA detection rate increases from 0% to 80% for samples collected >2 months after surgery. Second, they show that only patients who persistently cleared ctDNA during and after ACT did not relapse. Third,

they show that serial ctDNA analysis increases the accuracy of ctDNA to discriminate relapsing from non-relapsing patients, and that ctDNA detects relapse with a median lead time of 9.8 months compared with CT imaging. Finally, the authors establish two subsets of patients according to ctDNA growth rate, which strongly correlates with relapse and survival.

Several important questions, thoughts and recommendations arise from this publication. First, from a practical point of view with direct clinical implications, the study gives insights on recommendations regarding the best time-point for blood extraction which is established around 8 weeks after surgery, as well as the recommendation to repeat ctDNA assessments every 3 months to increase the accuracy of ctDNA to detect MRD. Moreover, serial ctDNA samples allow for characterization of dynamics and growth rate which have prognostic implications, although it needs to be further validated. Second, from a technical point of view, ctDNA MRD applications are enabled by very high positive predictive value for recurrence in patients with positive ctDNA. This is why it is crucial to use ctDNA assays specifically designed for MRD application. The authors use a validated strategy based on the identification of tumor-specific somatic variants by next-generation sequencing of the surgical specimen and exploit these to monitor MRD non-invasively in the blood. This tumor-informed approach, together with filtering of CHIP variants, improves the accuracy of ctDNA to detect MRD. Plasma-only MRD detection strategies combining epigenomics and genomics have recently also demonstrated favorable results for MRD testing. Third, from a biological point of view, this study confirms that ctDNA positivity is not a marker of a high risk of recurrence, but rather defines molecular persistence of disease. Thus, we should change our nomenclature and consider stage III ctDNA-positive patients after definitive interventions as stage IV MRD. And finally, this study opens the possibility for the use of ctDNA dynamics as a surrogate endpoint for ongoing and future prospective clinical trials. ctDNA will indeed change our approaches for adjuvant therapeutic strategies; the clinical implementation will come by the hand of ongoing prospective clinical trials that show the clinical impact of ctDNA in patient's survival and set a new standard-of-care for stage III colon cancer patients.

**SINGLE-CELL ANALYSIS OF HUMAN NON-SMALL-CELL LUNG CANCER LESIONS REFINES TUMOR CLASSIFICATION AND PATIENT STRATIFICATION**

Immunotherapy has recently revolutionized non-small-cell lung cancer (NSCLC) treatment. Beyond programmed cell

death protein 1 expression and the potential role of tumor mutational burden (TMB), no other predictive biomarkers of response to checkpoint inhibitors (CPIs) have been clearly identified.<sup>4</sup> There is a need, however, for a better understanding of the immune-stimulatory versus immunoregulatory presentation of tumor-associated antigens, as well as parsing the tumor-related effects on tissue-resident and migratory innate cell types. In particular, very little is known about the role of the tumor genotype in determining response.

In an interesting paper, recently published in *Cancer Cell* by Leader et al.,<sup>5</sup> the authors, deeply studied 361 929 single cells from 35 early-stage NSCLC lesions by using single-cell RNA analyses. The authors integrated the results of single-cell RNA sequencing (scRNA-seq) of immune cells with cellular indexing of transcriptomes and epitopes by sequencing (CITEseq; 3), which allows the evaluation of both scRNA-seq and multiplexed single-cell surface protein measurements.

By this integrating analysis, the authors were able to reveal a pattern of inter-tumor variability, which was validated in multiple bulk RNA datasets and correlated with TMB and tumor driver mutations across multiple tumor types. In this experiment, the tumor samples of 35 NSCLC patients were analyzed and a specific active immunoprofile was detected and referred to as the lung cancer activation module (LCAM) consisting in PDCD1+CXCL13+-activated T cells, IgG+ plasma cells, and SPP1+ macrophages. This profile permitted splitting the population into LCAM high and low according to the immune activation.

In the analyzed cohort, none of the patients presented with STK11 mutations. TP53 mutant tumors correlated with an intensified LCAM response whereas KRAS mutation led to a diminished one. LCAM presence was found to be independent of overall immune cell content and correlated with TMB, cancer-testis antigens and TP53 mutations. High baseline LCAM scores correlated with enhanced NSCLC response to immunotherapy, even in patients with above median TMB, suggesting a potential predictive role of LCAM score. That measurement of LCAM may provide a more direct indicator of the immune system's propensity for checkpoint response. In conclusion, the authors could identify an immune activation signature, derived from scRNA-seq and CITE-seq analyses, that behaves as an integrator of tumor-associated antigen load and driver mutations, which is not related to overall immune content, but correlates with response to checkpoint blockade. LCAM confers a clear prognostic benefit in CPI treatment but not relative to chemotherapy.

### SINGLE-CELL ATLAS OF LINEAGE STATES, TUMOR MICROENVIRONMENT AND SUBTYPE-SPECIFIC EXPRESSION PROGRAMS IN GASTRIC CANCER

Gastric cancer (GC) is characterized by presenting a wide heterogeneity within and across patients. This heterogeneity also involves the tumor microenvironment, which plays a relevant role both in GC development and in resistance to

current therapies. Studying GC and the tumor immune cells distribution and function is crucial to better understand the GC landscape and its potential impact in clinical outcomes. In this way, scRNAseq is conceived as a powerful tool to characterize gene expression across thousands of cells, allowing the identification of different cell lineages in several biological states and conditions.

Although there are already some previously published investigations employing scRNAseq to study GC heterogeneity,<sup>6,7</sup> Kumar et al.<sup>8</sup> have recently reported in *Cancer Discovery*, the largest transcriptomic analysis at single-cell level to date. The authors from Patrick Tan's group at Dukes and National University in Singapore studied 48 samples—primary, metastatic and matched normal gastric tissue—belonging to 31 patients, ranging from stage I to IV and comprising distinct histological and molecular subtypes. scRNAseq revealed 34 unique tissue states grouped into five major cell types: epithelial, myeloid, lymphoid, plasma and stromal. This in-depth analysis allowed the discovery of a not previously described stromal cell population that expresses both endothelial (PLVAP) and fibroblast (RGSS5) markers, possibly emphasizing cells undergoing endothelial-mesenchymal transition.

Likewise, a new cancer-associated fibroblast (CAF) subtype was also identified. These CAFs overexpressed inhibin beta (INHBA) and the fibroblast activation protein axis. Survival analyses using samples from the Cancer Genome Atlas (TCGA) database revealed poorer survival for tumor samples with a high INHBA expression. Besides that, a comparative analysis between diffuse and intestinal tumor samples revealed a higher proportion of plasma cells in diffuse-type tumors. The authors proposed that these cells could have been recruited by Krüppel-like factor 2 (KLF2+) epithelial cells, according to other experiments including spatial transcriptomic assays. Finally, scRNAseq on four pairs of tumor and matched normal GC patient-derived organoids (PDOs), showed that tumor PDOs had increased transcriptional plasticity compared with normal PDOs, underlining this phenomenon as possibly responsible for GC intra-heterogeneity.

This work provides wide information about the tumor microenvironment composition in GC, describing diverse tissue lineage states and rare cell populations. The study allows new exploratory opportunities to elucidate whether plasma cell infiltration in diffuse tumors plays a protumoral or antitumoral role in GC. Interestingly regarding the CAFs population described, INHBA appears as a novel potential therapeutic target. Finally, this work also consolidates PDOs as a valuable tool to study molecular mechanisms driving tumor heterogeneity, as well as to guide precision and personalized medicine for GC patients.

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## REFERENCES

- Henriksen TV, Tarazona N, Frydendhal A, et al. Circulating tumor DNA in stage III colorectal cancer, beyond minimal residual disease detection, towards assessment of adjuvant therapy efficacy and clinical behavior of recurrences. *Clin Cancer Res*. 2021. <https://doi.org/10.1158/1078-0432.CCR-21-2404>. In press.
- Reinert T, Henriksen TV, Christensen E, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncol*. 2019;5:1124-1131.
- Tarazona N, Gimeno-Valiente F, Gambardella V, et al. Targeted next generation sequencing of circulating-tumor DNA for tracking minimal residual disease in localized colon cancer. *Ann Oncol*. 2019;30:1804-1812.
- Remon J, Passiglia F, Ahn MJ, et al. Immune checkpoint inhibitors in thoracic malignancies: review of the existing evidence by an IASLC expert panel and recommendations. *J Thorac Oncol*. 2020;15:914-947.
- Leader AM, Grout JA, Maier BB, et al. Single-cell analysis of human non-small cell lung cancer lesions refines tumor classification and patient stratification. *Cancer Cell*. 2021. <https://doi.org/10.1016/j.ccell.2021.10.009>. In press.
- Derks S, de Klerk LK, Xu X, et al. Characterizing diversity in the tumor-immune microenvironment of distinct subclasses of gastroesophageal adenocarcinomas. *Ann Oncol*. 2020;31:1011-1020.
- Zhang M, Hu S, Min M, et al. Dissecting transcriptional heterogeneity in primary gastric adenocarcinoma by single cell RNA sequencing. *Gut*. 2021;70:464-475.
- Kumar V, Ramnarayanan K, Sundar R, et al. Single-cell atlas of lineage states, tumor microenvironment and subtype-specific expression programs in gastric cancer. *Cancer Discov*. 2021. <https://doi.org/10.1158/2159-8290.CD-21-0683>. In press.