Enumeration of CK+ cells with the nCyte Dx® system and CK-19 RT-qPCR

Microscopic enumeration of cytokeratin (CK)-positive cells in Small Cell Lung Cancer (SCLC) using the nCyte Dx® system and evaluation of results with real-time quantitative PCR (RT-qPCR)

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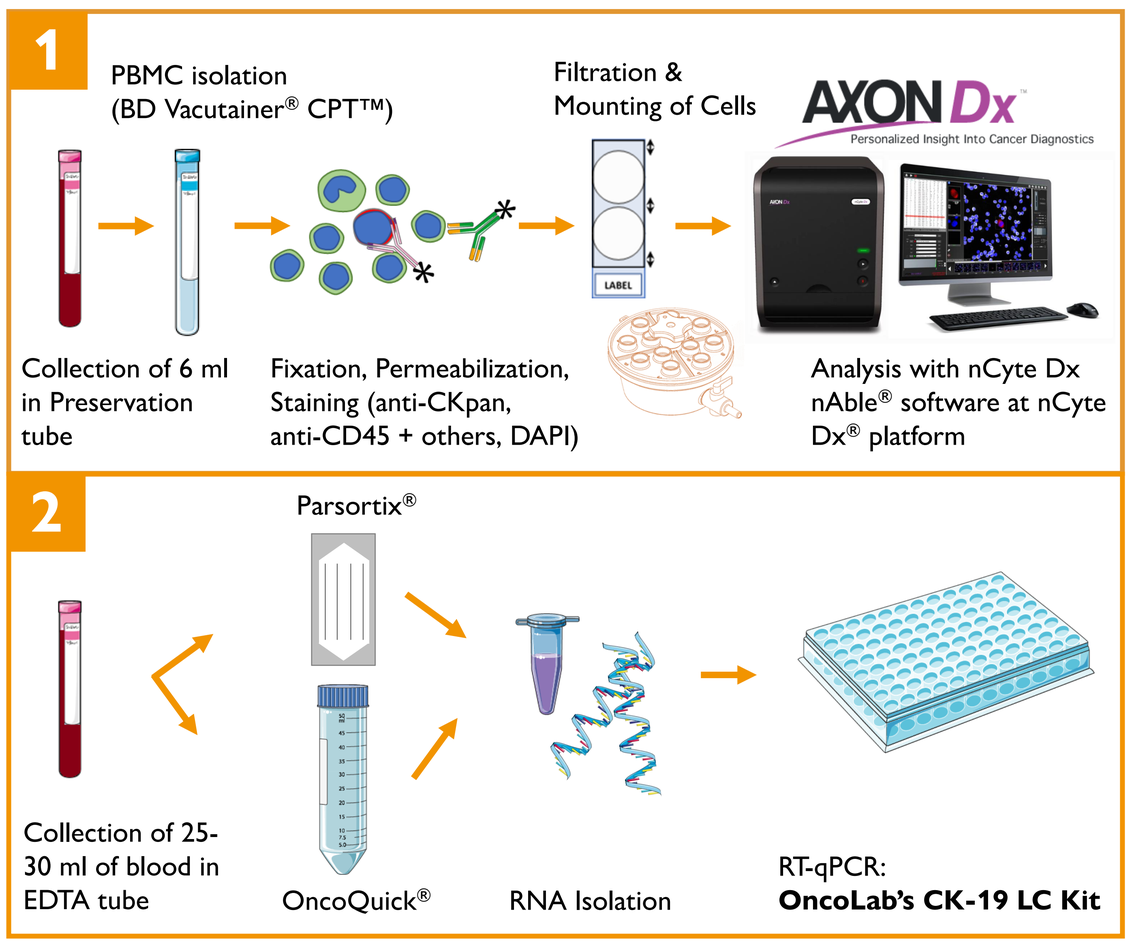
BACKGROUND

The identification of CTCs as a prognostic marker is generally accepted and their use in future diagnostic tools is under constant investigation. Different methods for CTC diagnostics have their pros and cons. While the information on morphology, i.e., apoptotic characteristics or cluster formation, is a major advantage of immunofluorescence staining, RT-qPCR enables the investigation of several genes of interest in parallel.

The TNM (tumor-node-metastasis) classification is a system to describe the amount and spread of cancer in a patients' body. Two out of 3 patients are diagnosed with extensive disease at initial diagnosis (1).

Here, we stained blood samples from SCLC patients with the **nPAC™ CTC IF Kit** and analyzed those samples with the AI-based **nCyte Dx nAble® software** at the **nCyte Dx® platform** (Axon Dx, LLC). In parallel, blood samples were processed with Parsortix® and OncoQuick®, and gene expression of CK-19 was analyzed using **OncoLab's CK-19 LC Kit** (2).

METHODS



RESULTS

MICROSCOPIC ENUMERATION OF CK+CD45- CELLS YIELDS THE HIGHEST NUMBER OF CK+ SAMPLES

Table 1: Enumeration of positive (orange) and negative (grey) samples by immunofluorescence staining and RNA analysis.

Patient Nr.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
nPAC™ CTC IF Kit	CK+CD45- cells	0	2	11	3	918	8	2	809	2	9	9	0	1	0	14	8	5	10	1	0	3	16	2	31	19
	Cluster	0	0	0	0	9	0	0	14	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	4
CK-19 LC Kit	Parsortix®	neg	neg	pos	pos	neg	neg	neg	pos	pos	pos	neg	neg	neg	neg	neg	pos	neg	pos	pos	neg	neg	pos	neg	pos	pos
	OncoQuick®	neg	neg	pos	neg	neg	neg	neg	pos	neg	neg	neg	neg	neg	neg	neg	neg	pos	pos	neg	neg	neg	pos	neg	pos	pos

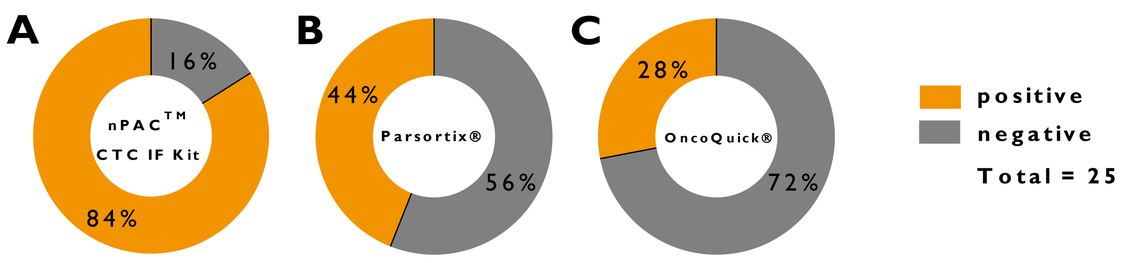


Figure 1: Percentage of positive and negative samples analyzed by immunofluorescence microscopy or RT-qPCR. **(A)** Blood samples were stained with the nPAC™ CTC IF Kit and analyzed at the nCyte Dx® platform. **(B)** Size-dependent enrichment of blood samples by Parsortix® technology. **(C)** Isolation of PBMCs with OncoQuick® tubes. **(B+C)** RNA isolation was performed with RNeasy Micro Kit (Qiagen). Gene expression of CK-19 was analyzed, and GAPDH was used as reference gene. None of the 11 tested control samples showed gene expression of CK-19.

THE NUMBER OF DETECTED CK+CD45- EVENTS IS ASSOCIATED WITH INITIAL TNM STAGING

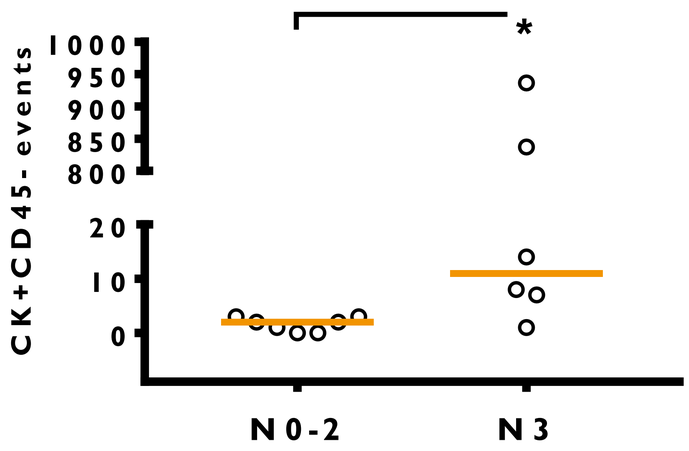
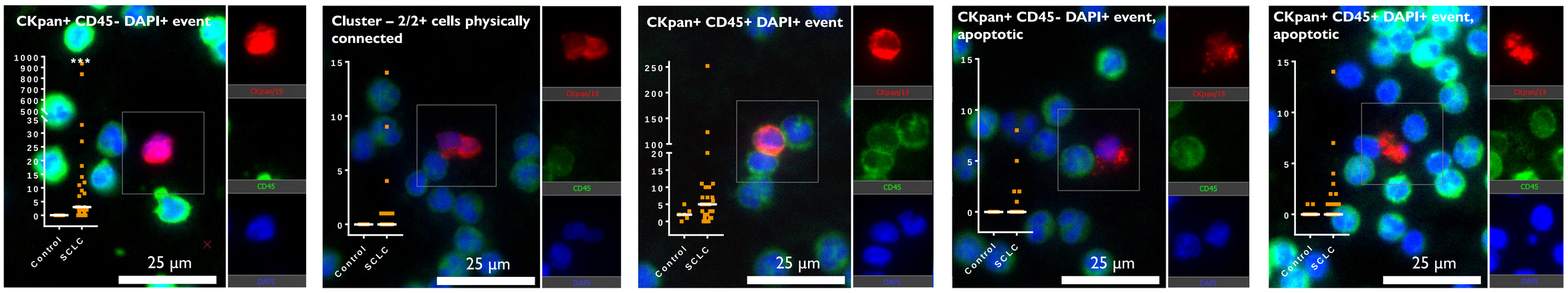


Figure 2: Higher numbers of CK+ CD45- cells are detected for the nodal status N3. CK+CD45- cells were microscopically enumerated by the nCyte Dx® system. Graph displays medians. Statistically significant differences are calculated by Mann-Whitney test (*p=0.0157). N=7 (N0-2); N=6 (N3).

CHARACTERIZATION OF EVENTS // EXAMPLES (in 6 ml of blood)



CONCLUSION

Conclusively, the nPAC™ CTC IF Kit, combined with the nCyte Dx nAble® software and the nCyte Dx® platform, showed to be a promising superior tool to quantify CK+CD45- cells in peripheral blood samples from SCLC patients in this study.

CK-19 RT-qPCR results obtained from Parsortix® enriched samples matched the results obtained from the **nCyte Dx® platform**. However, more CK+ samples were detected with the **nCyte Dx® system**. It needs to be clarified if immunofluorescence microscopy of Parsortix® enriched samples would lead to comparable results as those generated by the AI-based **nCyte Dx nAble® software.**

[1]American Cancer Society®, 2021, *Small Cell Lung Cancer Stages*, viewed on 14/09/21, 10:00 am, https://www.cancer.org/cancer/lung-cancer/detection-diagnosis-staging/staging-sclc.html
[2]This study received support from Angle plc. (UK) in the form of an in-kind contribution of Parsortix™ devices and microfluidic separation cassettes.

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