Vortex

White Paper

Vortex Uniquely Positioned in Liquid Biopsy Market to Deliver CTCs for Analysis

Dr. Elodie Sollier, Vortex Co-Founder

Stefan Nahum, EMV Capital Healthcare Analyst

Vortex Biosciences
Innovation Gateway
The London Cancer Hub
Sutton, UK

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Summary

Collecting circulating tumour cells (CTCs) from a tube of blood provides a sample representative of all tumour cell populations, and can be collected frequently, providing real-time monitoring of tumour evolution, treatment effectiveness, and cancer metastatic risks.

The Analysis of CTCs and ctDNA are complementary rather than mutually exclusive. Companies such as GRAIL Inc., who focus on ctDNA analysis, are driving the growth of the liquid biopsy market enhancing the environment for companies such as Vortex, to grow and prosper.

Vortex' VTX-1 platform has been in use as a Research Use Only instrument at multiple sites around the globe. It has been characterised and validated for CTC isolation from blood samples of metastatic cancer patients with breast, colon, lung and prostate cancer.

Numerous downstream assays have been developed with the VTX-1 for various research applications, including: immunofluorescence staining for CTC enumeration and characterisation, functional assays, cytopathology and cytogenetics, Sanger sequencing, RT-qPCR, qPCR, next-generation sequencing, and Western-blotting on single cells.

To address R&D market needs, Vortex is developing fully integrated workflows for the isolation of CTCs from cancer patient samples and their subsequent characterisation by single-cell RNA sequencing combining Vortex VTX-1 with 10x Genomics Chromium, or by collection, staining, and enumeration on a glass slide by combining Vortex VTX-1 with scanning platforms. The VTX-1 system has also demonstrated its ability to isolate circulating foetal cells from maternal blood samples.

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1. Blood Liquid Biopsies

Cancer is the second-leading cause of death in the world, with over 19 million new cases recorded worldwide in 2020 [1]. Clinicians continue to rely on invasive tissue biopsies to assess a patient's disease and prescribe appropriate treatment regimens. Tissue biopsies, as a medical procedure, carry some risks, are expensive, and provide a limited description of the cancer. Single site tumour biopsies may not be representative of intra-tumour heterogeneity and may fail to reflect a patient's genetic diversity. Furthermore, as a patient undergoes treatment, their cancer evolves. Monitoring the cancer allows for detection of new mutations and adjustment of treatments.

Blood liquid biopsies focus on the sampling and analysis of different blood components [2]. Each component or analyte can provide uniquely valuable insights into a cancer patient's tumour profile, from early diagnosis to disease progression, treatment efficacy and relapse detection: circulating tumour DNA (ctDNA) is tumour derived fragmented DNA present in the bloodstream which has been shed from dead tumour cells. Circulating tumour cells (CTCs) are live or apoptotic cancer cells released from both primary and metastatic tumours, spreading in the body to create metastasis (Figure 1). CTCs are considered the "seeds" of metastasis, which causes 90% of cancer deaths. However, CTCs are extremely rare and surrounded by millions of blood cells, making their isolation extremely challenging. However, isolating CTCs from a tube of blood provides a sample representative of all tumour cell populations, and can advantageously be frequently repeated, providing real-time monitoring of tumour evolution, treatment effectiveness, and cancer metastatic risks [3].

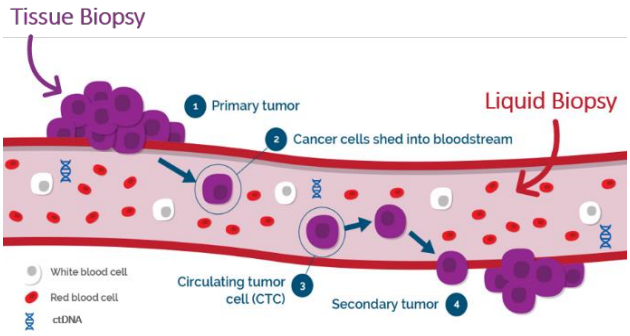

Figure 1: CTCs at the source of the metastasis.

2. Current Market Dynamics

The liquid biopsy market is currently centred on the high throughput and scalable molecular tests which focus on ctDNA. Such tests can be carried out to screen large cohorts in a short amount of time. For example, in the UK, the NHS has announced plans to make use of GRAIL's cancer spotting blood test in 165 000 patients. Companies such as GRAIL Inc. are driving the growth of the liquid biopsy market and enhancing the environment for companies such as Vortex, which focus on complementary analytes like CTCs, to grow and prosper. Vortex is one of several mature players in the CTC marketplace and has a unique differentiator: its ability to deliver live and label free CTCs for a wide range of analytical techniques with unparalleled efficiency and purity.

To isolate CTCs, some technologies target epithelial cell surface biomarkers such as EpCAM. Among these technologies are CellSearch [4], Adnagen [5], MagSweeper [6], and Fluxion [7]. By excluding cells lacking these markers, label-based approaches miss key CTC subtypes and provide limited insight into cancer heterogeneity [8]. Furthermore, label-based approaches lack transferability to multiple cancer types, for which no universal surface marker is available. Despite these limitations, the CellSearch system remains the only CTC platform approved by the FDA for clinical indication [9, 10], specifically for CTC enumeration as a means of prognosis for prostate, colon, and breast cancers.

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As an alternative, some CTC enrichment technologies called "label-free" rely on the small differences in size between CTCs and surrounding blood cells for their separation [11]. Microfilter technologies, like Parsortix from Angle [12], ScreenCell [13], ISET from RareCells [14] or CellSieve™ [15], flow blood through microscale features, pores or constrictions, to selectively trap larger CTCs. While this is reasonably efficient for on-chip staining and enumeration, such processes suffer from the off-chip release of trapped cells for downstream analysis. To avoid such limitations, ClearCell FX from Clearbridge uses continuous Dean flows in a spiral channel. However, this requires an RBC lysis step before CTC isolation: a process that requires manual intervention and chemical reagents that can negatively impact the target CTCs [16].

Thus, several technologies have been developed for isolating CTCs [17], each with specific pros and cons for commercial traction. There remains an unmet need for a simple, label-free, and fully automated capture of intact CTCs, with cells released in suspension in a format compatible with various downstream assays. We believe that the Vortex VTX-1 – further described later on - is the most flexible, technologically advanced, and efficient instrument on the market to enable the most comprehensive range of downstream analyses to satisfy this unmet need.

To illustrate this, Prof. Stuart Martin of the University of Maryland concluded in a paper in which he and his team tested the integration of various CTC isolation technologies with their TetherChip technology, that they "*ultimately prioritised the Vortex VTX-1 platform in this study due to its quick isolation processing time, minimal cell manipulation as well as ease of directly harvesting viable cells onto the TetherChip for downstream analysis*" [18].

3. Value of CTCs vs ctDNA

CTCs and ctDNA are sometimes considered to be competing with each other (Figure 2), with ctDNA ahead of the battle for 3 key practical reasons [2, 3]:

1. *ctDNA clinical adoption is further advanced*, as ctDNA is easier to extract from a blood sample, with a simple centrifugation step to extract the plasma. This 'head start' has benefited the early adoption of ctDNA in liquid biopsy workflows.

2. *ctDNA can be readily shipped or stored after its isolation and before analysis*. This is particularly important in clinical trials where batch sample processing may be preferred. Conversely, CTCs are fragile, and shipping blood samples can affect their integrity and successful isolation. However, dedicated blood collection tubes have recently been developed to preserve the cells and allow the shipping of blood samples for up to five days after the collection [19].

3. *Downstream analysis of ctDNA is also significantly more advanced*, with assays commercially available and clinically approved. For example, EGFR mutation profiling on ctDNA from non-small cell lung cancer (NSCLC) patients, with the Roche Cobas or the Qiagen Therascreen assays, can be used at the time of diagnosis or during cancer recurrence to identify drug-resistance-conferring mutations and adjust the patient's treatment regimen accordingly [20]. Another example is the Guardant 360 test, the first FDA-approved test for all advanced solid tumour s to provide the broad analysis of 73 gene mutations to help guide the selection of optimal treatment options for patients [21]. Another example is the ctDNA methylation marker analysis [22] covered, for example, by the GRAIL Inc. Galleri test for early detection of cancer.

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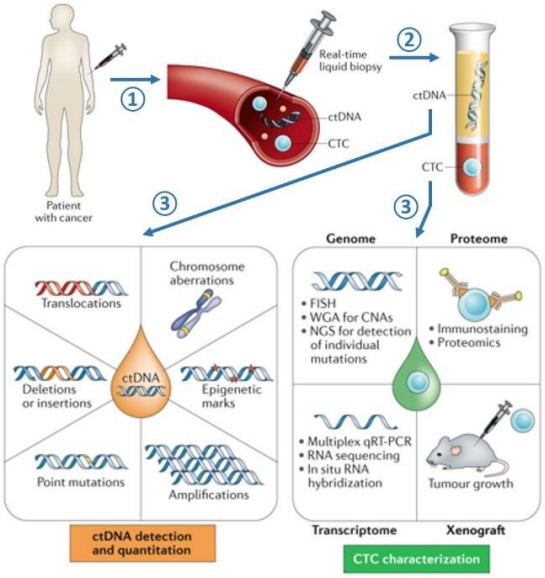

Figure 2: CTCs and ctDNA; may have the same origin but distinct and complementary uses. Modified from https://doi.org/10.1038/s41571-019-0187-3.

We, and other industry players, strongly believe that CTCs and ctDNA offer distinct but complementary information to characterise cancer biology [3, 23].

While ctDNA analysis focuses on small DNA fragments shed from dead cells, CTCs are living cells that provide a range of additional analytical targets, such as RNA, proteins, functions, that can recapitulate the cancer biology more comprehensively. Among the various analysis techniques that are only possible with the CTCs, we can cite:

1. *RNA expression analysis.* Several RNA-based assays are moving towards clinical use, with the best example being AR-V7 RNA expression analysis in metastatic castration-resistant prostate cancer patients for treatment selection [24, 25].

2. *Protein expression, as potential companion diagnostic test*. PD-L1 expression in CTCs indicates a poor prognosis for NSCLC and head/neck patients [26, 27].

3. *Live CTC assays*. At the point of biopsy, CTCs may be living cells [28]; they can be cultured, analysed for secretions, or inoculated into mice to generate patient-derived xenografts, mouse models that are more representative of a patient's cancer. These in vivo models can, in turn, help understand tumour biology and predict the therapeutic response of the patient, all in a research setting [29; 30].

4. *Cytology and cytogenetic analysis.* FISH and cytology analysis on tumour tissue cells are part of the current standard of care, looking at specific genomic mutations or amplification for companion diagnosis (ALK rearrangements or HER2 amplification for example) or morphological features respectively [31]. These analyses can also be performed on CTCs as the sample, with such assays subject to regulatory approval before a clinical usage.

5. *CTC Enumeration* has shown correlation with patient early cancer diagnostics, prognosis, treatment monitoring and early recurrence detection. The FDA-approved CellSearch instrument has helped promote CTC enumeration as a biomarker [9, 10].

For all these reasons, CTCs and ctDNA should be perceived as complementary rather than exclusive to one another, for a broader understanding of each patient's cancer and better treatment throughout the cancer care.

Several publications have highlighted the concept of total liquid biopsy, or multimodal strategy, with the isolation and analysis of CTCs and ctDNA [32, 33, 34]. For example, Sundaresan *et al.* at the Massachusetts General Hospital demonstrated the benefits of looking at CTCs as well as ctDNA for the T790M EGFR mutation profiling [32]: *"The use of complementary approaches may provide the most complete assessment of each patient's cancer (for T790M mutation detection)."* Similarly, Keup *et al.* compared ctDNA and CTC gDNA in metastatic breast cancer patients and suggested *"a coordinated analysis of both fractions in order to provide a comprehensive genomic footprint that may contribute to identifying the most suitable therapy for each individual"* [34].

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4. Vortex VTX-1 System Features & Performance

Figure 3: VTX-1 System Features & Technology.

With the Vortex VTX-1 system, CTC capture is based on cell size, shape and deformability, **independently of tumour biomarkers**. The Vortex technology exploits inertial microfluidics and laminar microscale vortices to isolate larger CTCs from smaller blood cells [35]. The Vortex microfluidic chip consists of 16 parallel channels and 9 serial reservoirs in each channel [36]. The single use Vortex Cartridge, which contains the chip, the collection container and the blood collection tube, is inserted into the VTX-1 Liquid Biopsy System. The user interface is extremely simple, enabling the user to select the desired protocol and start a blood run. The diluted blood is automatically injected through the microfluidic chip for CTC capture, while the flow-through is collected in the recycling tube. Once the entire volume of blood has been processed and blood cells have been washed out, the flow rate is lowered and the vortices dissipate. In this way, captured cells can be released off-chip directly into the collection container.

Performance

Depending on the operation mode selected by the user, the blood is transferred from the recycling tube and re-processed through the chip for a second and/or third cycle. By adjusting the number of cycles, it is possible to favour either an optimal cancer cell recovery ("high recovery mode", 3 cycles) or a better purity ("high purity mode", 1 cycle) [36]. When processed with the high purity mode, 53.8% of MCF7 cells were collected on average, for only 101 contaminating WBCs/mL of blood processed. When the same sample was processed with a high recovery mode (three cycles), 71.6% of the cells were recovered, for 350 contaminating WBCs/mL. The repeatability of the capture efficiency further underlines the reliability of the technology with standard deviations from 6.5% to 8.6% depending on the protocols [36].

Technological Advantages

The isolation process does not require filters that can get clogged or retain cells and allows for optimal CTC recovery with a very low WBC contamination, while maintaining the intact and viable cells, for broad access to cellular content. The isolation is label-free, with no need for antibodies, chemistry, or difficult-to-scale processes. The instrument is fully automated, and blood is self-contained, leading to a high level of reproducibility and safe usability, thus being accessible to clinical labs. Finally, the cells can be easily released into different containers, depending on the downstream assay, giving the user a high level of flexibility for the CTC characterisation downstream. The Vortex technology is the ideal approach for isolating cancer cells from blood, with key product features to ultimately help researchers, clinicians and cancer patients at different stages of the cancer care pathway.

5. Vortex VTX-1 Compatibility with Downstream Assays

The Vortex technology has been characterised and validated for CTC isolation from blood samples of metastatic cancer patients with breast, colon, lung, and prostate cancer. Numerous workflows have been optimised for various CTC research applications, such as immunofluorescence staining and CTC enumeration [35, 37-39], functional assays [18, 40], cytopathology and cytogenetics [41], Sanger sequencing [42], RT-qPCR, qPCR [43], next generation sequencing [44, 45] and even Western blotting on single cells [46]. The users can collect the isolated CTCs in their container of choice depending on the downstream assay they wish to undertake.

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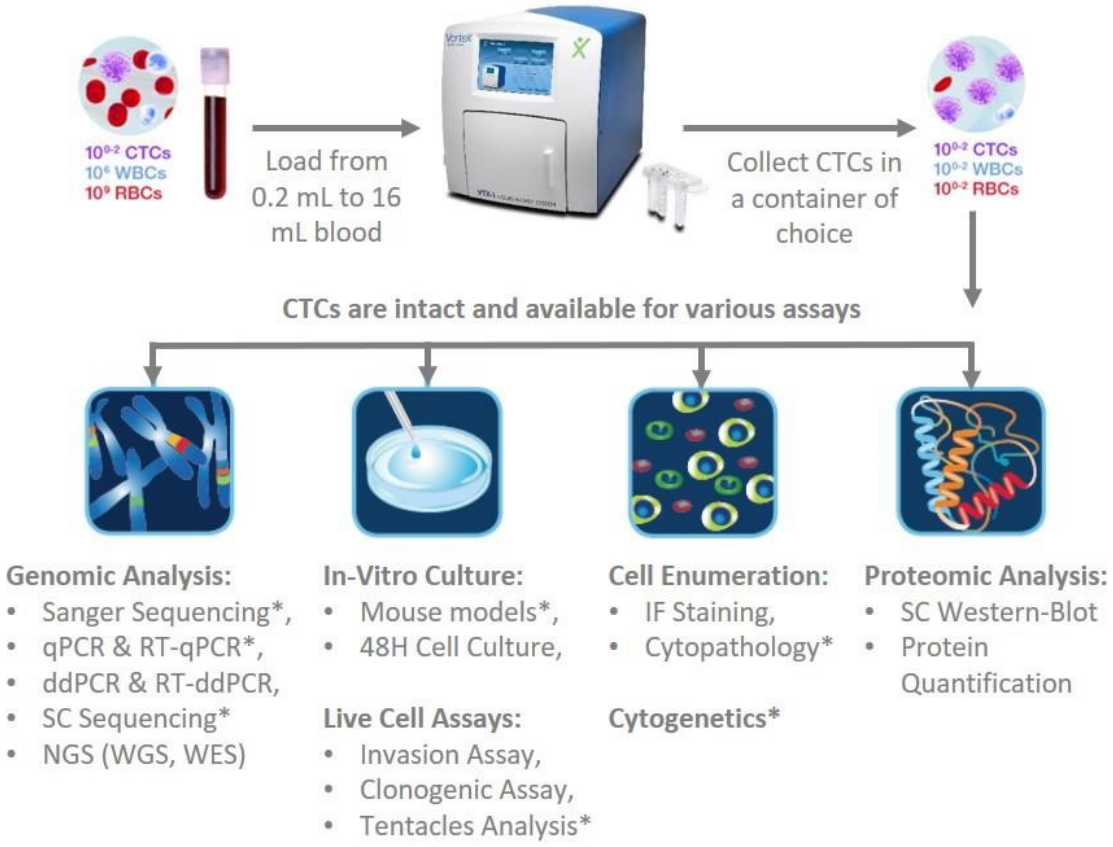

Figure 4: Downstream Applications for CTCs. Workflows indicated with * have been shared with the community through Vortex application protocols, application notes or peer-reviewed publications.

Downstream Assays and Link to Vortex Biosciences Application Notes:

a. CTC PCR-based Sanger Sequencing

- Mutation profiling is crucial to guiding treatment decisions and is currently completed using tumour biopsy samples, an invasive process that can be limited by small sample size and difficult access to the tumour site. CTCs may be used in place of tumour biopsy for mutation profiling but require a significant enrichment to high levels of purity for DNA extraction and Sanger sequencing.
- CTCs collected with Vortex technology were seamlessly integrated within a genomic workflow, i.e. DNA extraction, PCR, and Sanger sequencing. The high purity of CTCs achieved by enrichment using the Vortex VTX-1 system makes it ideal for Sanger sequencing assays. This protocol can be adapted to other gene mutations as well.
- Our workflow and primers were validated with cancer cell lines spiked in whole blood, processed through Vortex, and DNA mutations detected afterward, from DNA input as low as 20 pg and DNA purity >10%.
- This workflow was applied on colorectal cancer patients, with comparison of the mutations between CTCs, liver tissue and FFPE samples from the same patient. No mutation was detected in age-matched healthy donors (N=10) [42].

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Donor ID	Time Point of Collection	KRAS				BRAF				PIK3CA				CTCs/ml	Purity %
		Primary	Liver Met	CTCs	ctDNA	Primary	Liver Met	CTCs	ctDNA	Primary	Liver Met	CTCs	ctDNA		
P001	Pre-Op		G12D				ND				ND				
	Follow up 1			ND	ND			ND	ND			ND	ND	0.1	0.6
	Follow up 2			G12D	ND			ND	ND			ND	ND	7.2	39.4
	Follow up 3			ND	ND			ND	ND			ND	ND	0.3	3.0
P006	Pre Op		ND	ND	ND		ND	V600E	ND		H1047R	H1047R	H1047R	3.8	23.8
	Follow up 1			ND	ND			ND	ND			H1047R	H1047R	25.0	54.6
	Follow up 2			ND	ND			ND	ND			H1047R	H1047R	29.0	63.5

Figure 5: (1) Workflow for the use of the VTX-1 Liquid Biopsy System for CTC PCR-based sequencing.
(2) KRAS, BRAF and PIK3CA mutation detection in frozen primary tumour (FFPE), frozen liver metastatic tissue, CTCs and plasma ctDNA in Colorectal cancer patients [42].

a. CTC Isolation and RT-qPCR

- A workflow was developed to perform gene expression analysis on VTX-1 isolated cells, i.e., including RNA extraction in-bulk, reverse transcription, cDNA pre-amplification and qPCR.
- This protocol was applied to the detection of PD-L1, a key factor in determining if PDL1 and/or PD-1 based immunotherapies will be effective. This protocol can also be adapted to other gene targets.

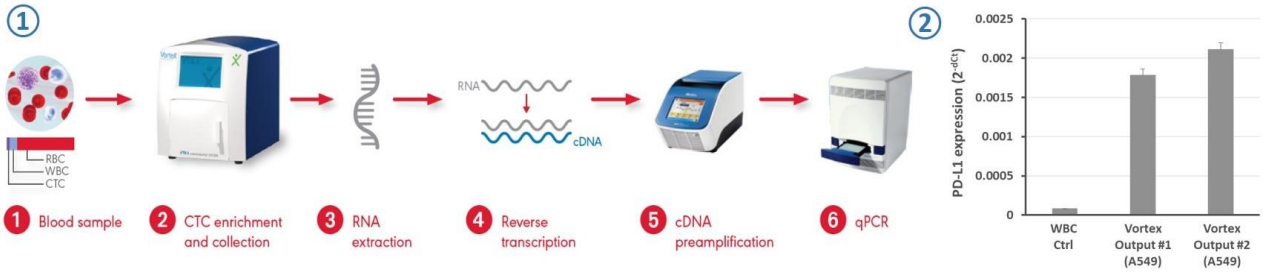

Figure 6: (1) Workflow for the use of the VTX-1 Liquid Biopsy System for CTC RT-qPCR analysis.
(2) Application to the evaluation of PD-L1 expression on CTCs, with A549 cancer cells spiked in blood (N=2 duplicates), processed through the VTX-1 and analysed by RT-qPCR for PD-L1.

b. Single-CTC RNA Sequencing on 10x Genomics Platform

- The VTX-1 Liquid Biopsy System serves as a rapid sample preparation platform to yield intact cells directly available and compatible for single-cell RNA sequencing on 10x Genomics Single-Cell system.
- Vortex Biosciences -10x Genomics integrated workflow allows for the identification of distinct gene expression features in Circulating Tumour Cells overcoming traditional hurdles of pooled cell analysis.

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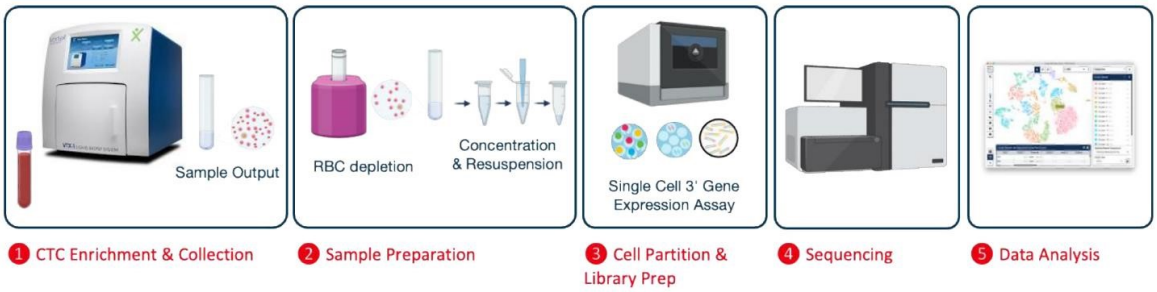

Figure 7: Workflow for the use of the VTX-1 Liquid Biopsy System in combination with 10x Genomics platform for single CTC RNA sequencing.

Evaluating the compatibility of the VTX-1 sample output with 10x Chromium Controller for Single-Cell Gene Expression Assays. To evaluate whether samples isolated with VTX-1 can interface seamlessly with the 10x Chromium workflow for single cell gene expression, blood from a healthy donor was spiked with lung cancer cell lines (HCC827 and H1975) and used as a surrogate for patient samples. Spiked-in blood samples were processed through VTX-1, the output samples post processed (as described in FIG 7) and loaded directly onto the 10x Chromium Controller for single-cell RNAseq using the Single Cell 3' Gene Expression Assay (v3).

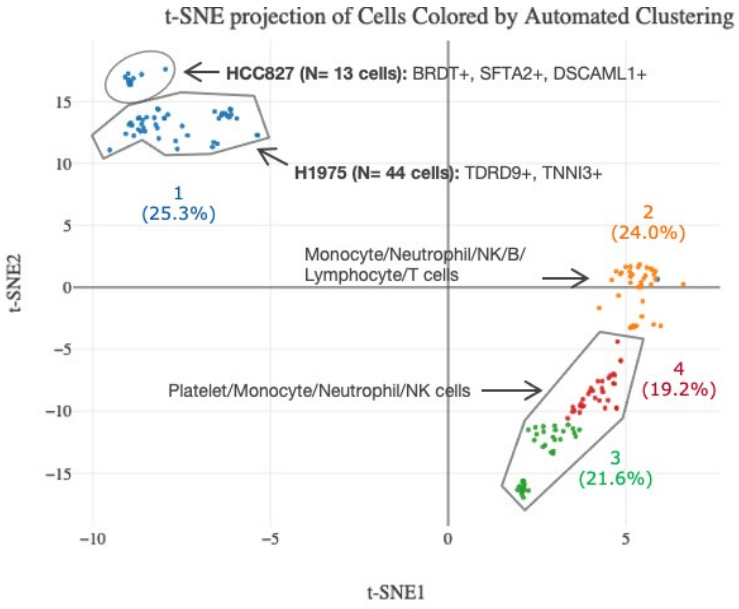

Figure 8: Distinct RNA signatures corresponding to two different non-small cell lung cancer cell lines are distinguished after single-cell RNA sequencing. In this experiment, blood from a healthy donor was spiked with HCC827 and H1975 in a 1:3 ratio (62 HCC827:197 H1975), processed with the VTX-1 and the output sample analysed with the Single Cell 3' v3 Gene Expression Assay. The t-SNE projection shows that the different cell populations present in the initial sample cluster distinctly and that cells with very similar gene expression profile can be successfully distinguished.

- Two non-small cell lung cancer cell lines (H1975 and HCC827) with similar gene expression signature spiked together in a blood sample cluster distinctly and can be successfully identified following single-cell RNA sequencing.
- The ratio between the two different lung cancer lines (13 HCC827:44 H1975 or about 1:3) is preserved which suggests there is no selection bias throughout the whole experimental process.
- The VTX-1 Liquid Biopsy System shows promising results as a rapid sample preparation platform to yield intact and viable cells directly available and compatible for single-cell RNA sequencing on 10x Genomics Single-Cell system.

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c. CTC Isolation from Mouse Models

- Vortex has developed a protocol for the isolation and characterisation of CTCs from a small volume of blood from mice xenograft cancer models using the VTX-1 Liquid Biopsy System.
- Users have demonstrated that blood collected through cardiac puncture is better suited to CTC studies, compared to blood from saphenous vein or retro-orbital sinus. This protocol was applied to the CTC isolation from patient-derived orthotopic xenograft (PDOX) mouse models generated from 7 triple-negative breast cancer (TNBC) patient samples [39, 47].
- The ability to interrogate CTCs from PDOX models is generating additional opportunities to understand cancer biology and to study therapeutic responses in in-vivo models.

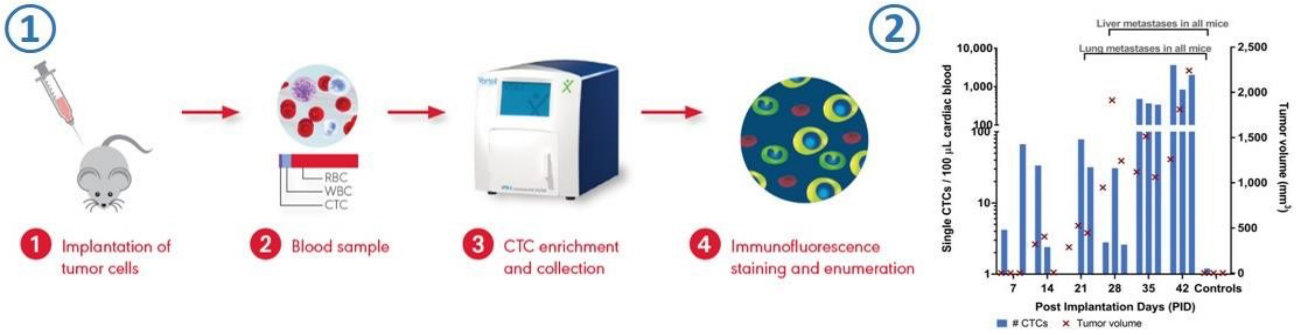

Figure 9: (1) Workflow for the use of the VTX-1 Liquid Biopsy System for CTC isolation from mice xenograft cancer models, immunofluorescence staining and enumeration. (2) Evolution of number of CTCs and tumour volume over time [39, 47].

d. CTC Microtentacle (McTN) Study with the TetherChip

- The direct integration of TetherChip technology with Vortex's CTC capture platform was pioneered by Pr S. Martin *et al*. from the University of Maryland and allows the study of CTC microtentacles within hours of isolation from the patient and without the need for long-term culture [18].
- This integrated workflow addresses the common challenges encountered when working with CTCs; cell loss, alteration of cell integrity, hands-on time and pre-processing steps.
- In proof-of-concept experiments, live cancer cells were successfully isolated from spiked blood samples, tethered onto a slide with a 95% efficiency, then stained, imaged, and their microtentacles analysed within 1 hour post isolation.
- This streamlined workflow opens possibilities for the clinical analysis of metastatic phenotypes for a wide range of cancer types, in real-time and throughout the patient's treatment.

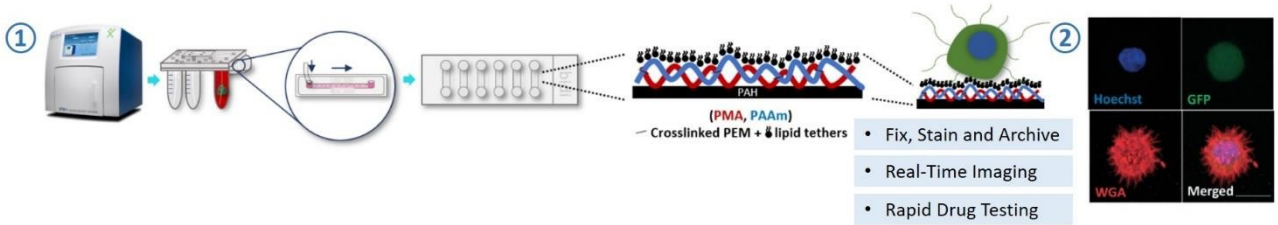

Figure 10: (1) Workflow for the use of the VTX-1 Liquid Biopsy System for CTC microtentacle analysis in combination with the TetherChip [18]. (2) MDA-MB-231 cells spiked into whole blood were isolated with the VTX-1, tethered onto a TetherChip surface and immunostained to visualise the microtentacles.

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e. CTC and Papanicolaou Staining

- Cytology staining such as the Papanicolaou (Pap) stain is a test routinely used for cancer diagnosis by yielding detailed information on nucleoli and chromatinic patterns of cells, sufficient to determine whether cells are benign or malignant.
- Vortex has developed a protocol for isolating human CTCs from blood and collecting them on a glass slide for cytology staining downstream.
- Using this workflow, CTCs isolated from 10 non-small cell lung cancer (NSCLC) and 6 breast cancer patient samples were collected and Pap-stained. Malignant cells detected in 9/10 and 6/6 cancer cases were compared to their corresponding primary tumour biopsies and revealed conserved morphological features [41].
- VTX-1 workflow is compatible with existing automated laboratory equipment and practices, making cytological investigation of CTCs a real possibility for ongoing disease monitoring.

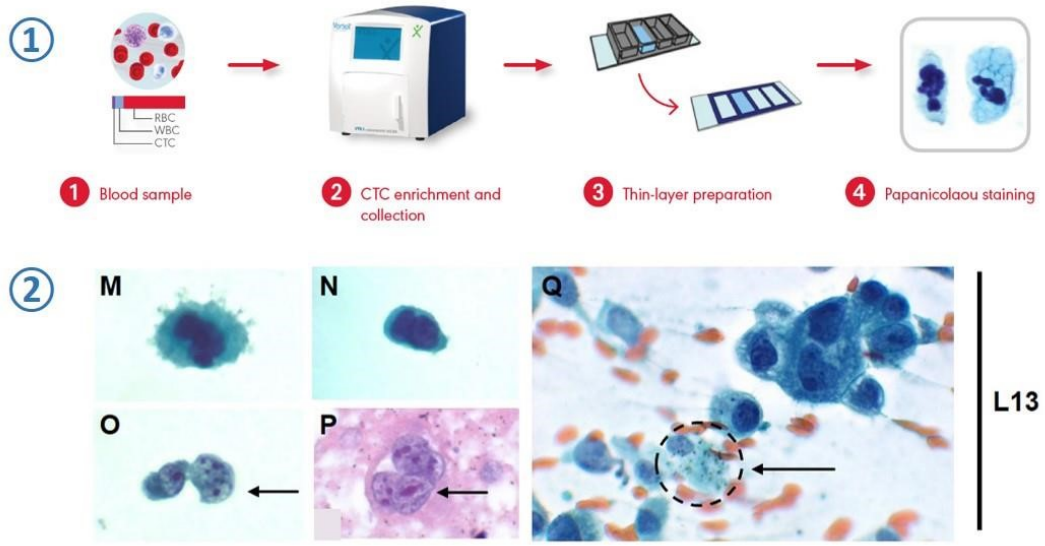

Figure 11: (1) Workflow for the use of the VTX-1 Liquid Biopsy System for CTC cytopathologic analysis. (2) Application to NSCLC patients and comparison to matched tumour biopsies. Cells isolated from the patient blood (M–O) had a very high N/C ratio and a distinct chromatin margination pattern that was recapitulated in cells from a Pap (P) and H&E stain (Q) of his fine needle aspirate [41].

f. CTCs and Fluorescence-In-Situ-Hybridization

- FISH analysis of CTCs can be a valuable non-invasive surrogate for routine tumour cytogenetic profiling and may provide additional information to help predict a patient's outcome, identify patients eligible for targeted drug therapy, and monitor response to treatment.
- Vortex has developed a protocol for isolating human CTCs from blood and collecting them on a glass slide for cytogenetic assay, supporting the compatibility of Vortex VTX-1 workflow with commercially available FISH assays. The high purity of the CTCs isolated from blood biopsies with Vortex VTX-1 is particularly well suited for cytogenetics studies, where high cellularity can sometimes confound analysis.
- The workflow was validated with cancer cell lines and probes for HER2/neu DNA and chromosome 17 centromere (PathVysion Kit, Abbott Molecular).
- Cytogenetic analysis of CTCs isolated from non-small cell lung carcinoma (NSCLC) metastatic cancer patients using the Vysis LSI ALK Break Apart Rearrangement FISH Probe Kit (Abbott Molecular) indicated similar fluorescence signal patterns between the CTCs and the primary tumour biopsy [41].

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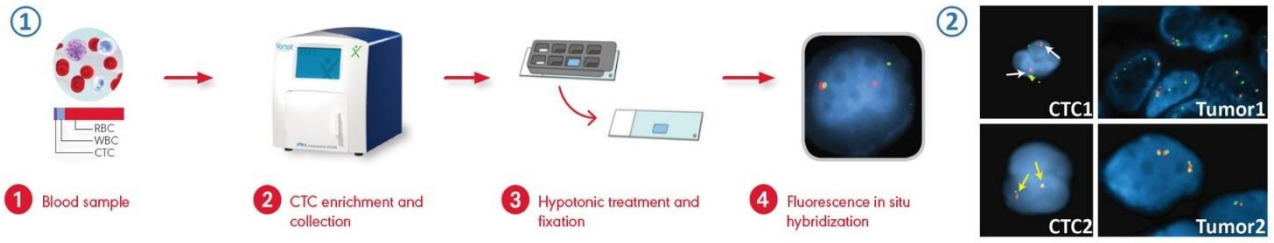

Figure 12: (1) Workflow for the use of the VTX-1 Liquid Biopsy System for CTC cytogenetic analysis. (2) Application to NSCLC patients and comparison to matched tissue biopsies. CTC1 & Tumour 1 belong to an ALK+ patient: Both CTCs and tumour patterns show ALK rearrangement and polysomy. CTC2 & Tumour 2 belong to an ALK- patient: Both CTCs & Tumour exhibit normal ALK signals [41].

6. Next Strategic Directions

a. Single Cell Analysis

To further address the R&D market needs, including the needs of pharmaceutical and biotechnology companies as well as academic research, one core strategic aim for Vortex is to develop fully integrated workflows for the isolation of CTCs from cancer patient samples and their subsequent characterisation by single-cell RNA sequencing. Vortex's technology offers the ability to combine the VTX-1 with the Chromium Single Cell Gene Expression solution from 10x Genomics, for single-cell resolution on a pooled suspension of cells [48], as well as with single-cell dispensing technologies, such as the cellenONE from Cellenion [49], for downstream RNA sequencing of single cells. Preserving the integrity and viability of sensitive samples such as CTCs during isolation and sample preparation is critical for gene expression analysis, and the success of single-cell RNA sequencing in particular. Building on our unique position of providing the highest quality viable CTCs, Vortex aims to position itself as the leading CTC isolation instrument to support downstream integration with multiple industry leaders in the Single-Cell Analysis arena.

b. Automated Imaging & Enumeration

Vortex technology was used successfully to isolate CTCs from blood samples of patients with metastatic breast (N=22), lung (N=15), prostate (N=20), and colon (N=41) cancer [41, 42, 44]. Figure 13 displays CTC enumeration data after immunofluorescent staining, imaging, and manual enumeration following published criterion for CTC classification [37]. Each dot represents the number of CTCs per 7.5mL of blood from one patient blood draw.

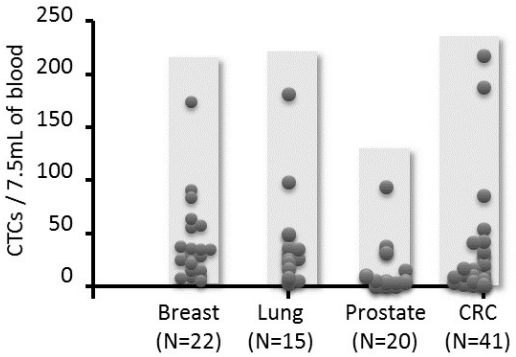

Figure 13: CTC counts from patient samples of various cancer types [41, 42, 44].

Circulating Tumour Cells (CTCs) offer a great potential to transform the standard of care for cancer patients. CTCs counts can help predict patient prognosis, monitor response to treatment and disease progression, and stratify patients in clinical trials. While the number of CTCs is associated with survival chances, the scoring of CTCs can be prone to inter-reader differences.

The combination of CTC isolation with an automated, high-resolution imaging system with advanced analytical capabilities is necessary to achieve objective scoring.

Toward this end, Vortex is developing a sample-toresult workflow, from CTC isolation to automated enumeration and characterisation, by combining Vortex's automated CTC isolation system with Axon Dx's nCyteDx® scanning platform.

The availability of standardised methodologies for cell isolation, staining, and imaging &

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enumeration is critical for clinical studies/trials performed across multiple clinical sites.

c. Other Applications: Foetal Cell Isolation and Maternal Blood Testing

Extremely rare cell populations such as circulating foetal cells are difficult to isolate with currently available technologies. In proof-of-concept experiments, the VTX-1 system's capabilities to isolate circulating foetal cells from maternal blood samples were evaluated in spike-in experiments with trophoblast cell lines. For these mock samples, the “circulating trophoblasts (CTs)” recovery was estimated to +/- 35%. In follow-up experiments with maternal blood samples, recovery of CTs was confirmed for 7 out of 10 first trimester samples from pregnant women carrying a male foetus. Between 2 and 6 CTs were detected from 8 mL of maternal blood, as estimated by a Y-chromosome-specific qPCR assay [50].

Foetal cells isolated and singled out from maternal blood samples using the VTX-1 could open new doors into research of foetal cell biology and downstream analysis of foetal cells. This will enable prenatal genomic-based testing to become an accessible option for expecting couples to make informed decisions on their pregnancy, as well as to consider pre-emptive decisions on the health of their neonate [51].

7. Conclusions

The authors’ strong opinion is that, as of today, both large corporate entities in the liquid biopsy and multi-omics space such as BD, Bio-Rad, Takara, Illumina and 10X Genomics, as well as rising companies including Freenome, Thrive, Biodesix and MissionBio already recognise the utility of CTCs and CTC-derived material (RNA, proteins, secretions, functions) as valuable analytes.

Vortex is at the forefront of CTC isolation. Analytical validation of VTX-1 performance is fundamental to consolidate Vortex’s position in the research market and ultimately establish its clinical validity through LDTs.

As leaders in the liquid biopsy space such as GRAIL Inc. achieve their vision of using blood biopsies for early cancer detection, with the ability to identify individual cancer tissue of origin, then cancer care pathways, screening techniques and treatments will be transformed, delivering new and enhanced opportunities for specialised market entrants, such as Vortex.

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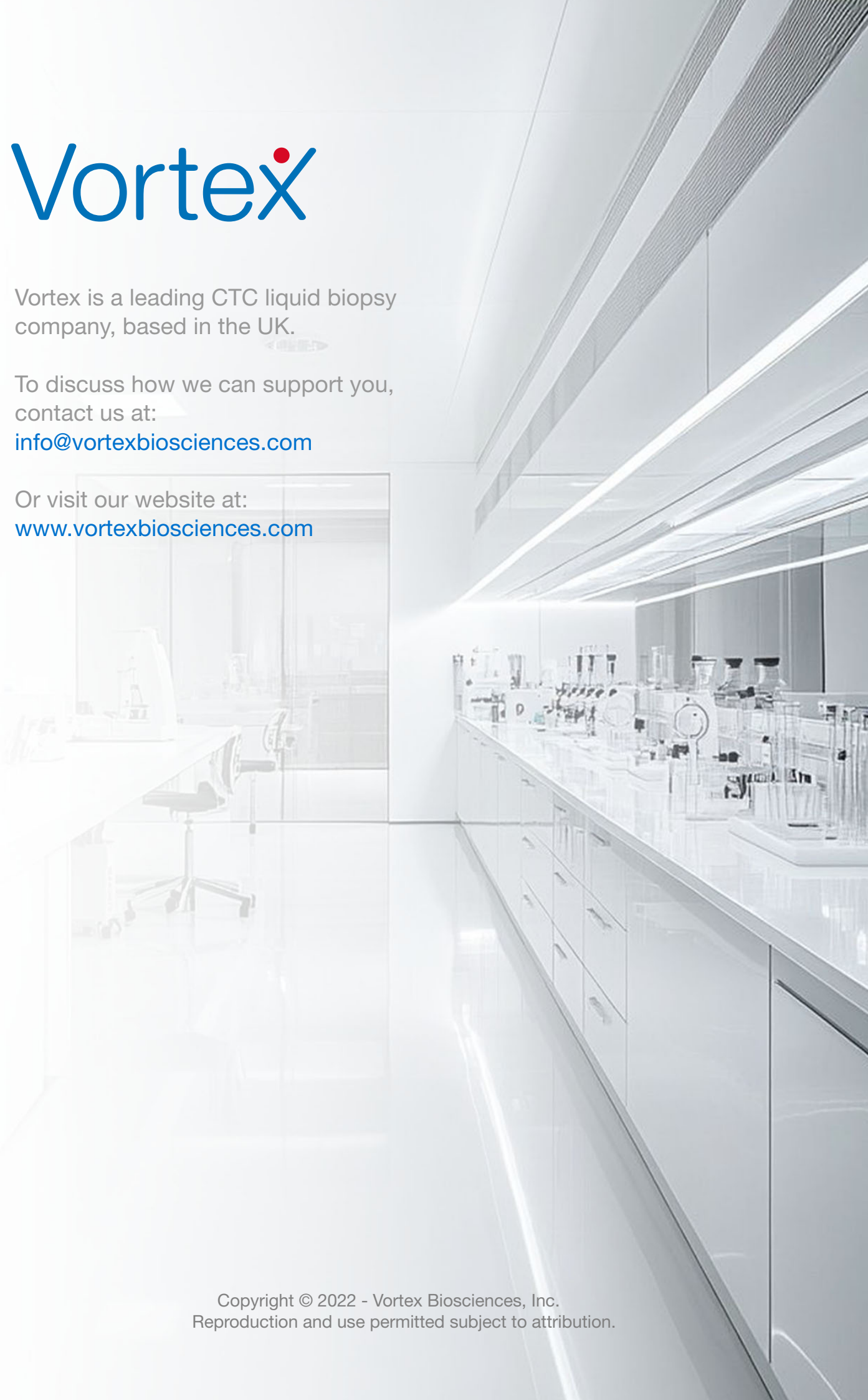

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