Workflow evaluation for molecular characterization of single circulating tumor cells in blood samples of patients with gynecological malignancies

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Background

We recently presented the feasibility of single circulating tumor cell (CTC) analysis using the QIAscout single cell isolation platform (Fig. Ⓐ, QIAGEN, Germany), followed by whole transcriptome amplification (WTA) and targeted Next Generation Sequencing (NGS).

Alternatively to the previously used single cell WTA – workflow, we here applied the QIAseq UPX 3' Transcriptome Kit (UPX) for high throughput gene expression analysis for single cell CTC analysis from blood samples of patients (pts) with metastatic breast cancer (MBC) and primary ovarian cancer (POC). This workflow has the advantage of better confirming the sequencing results using unique molecular identifiers (UMI).

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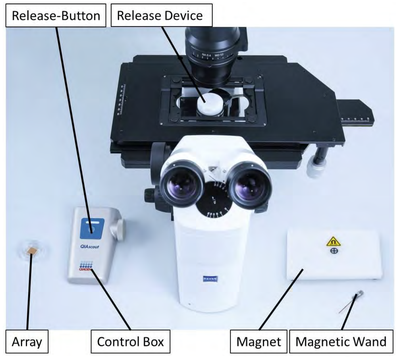

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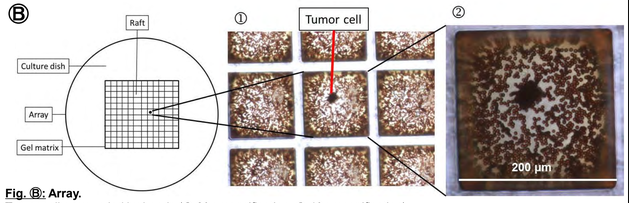

Fig. Ⓑ: Array.
Tumor cell surrounded by beads (① 20x magnification; ② 40x magnification).

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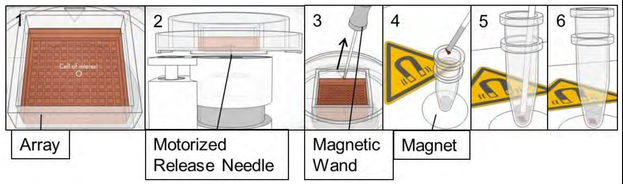

Fig. Ⓒ: Workflow for single cell isolation.

Updated Methods

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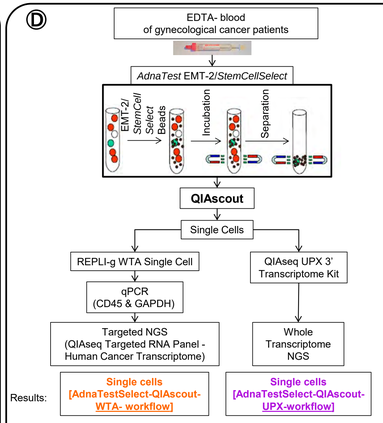

Experimental design (Fig. Ⓓ):
CTCs were isolated from 5 ml whole blood of 9 MBC, 2 POC pts and 2 healthy donors (HD) using positive immunomagnetic selection (AdnaTest *EMT-2/StemCell Select™*) followed by QIAscout single cell isolation. Adhesion of CTCs to the QIAscout array microrafts was supported by pre-treating the array with Corning® Cell-Tak™ Adhesive.
Using an inverted microscope, single cells were mechanically isolated by picking out one of the 12000 microrafts.
Subsequently, mRNA of single cells was amplified by WTA (REPLI-g WTA Single Cell Kit, QIAGEN) and cDNA of the cells was further analyzed by targeted NGS using a QIAseq™ Targeted RNA Panel (Human Cancer Transcriptome Panel, 395 genes) (Single cells [AdnaTestSelect-QIAscout-WTA-workflow]; n= 7 MBC) (data not shown).
QIAscout further allowed modified single cells analysis with the QIAseq UPX 3' Transcriptome Kit, followed by whole transcriptome NGS (Single cells [AdnaTestSelect-QIAscout-UPX-workflow]; n= 2 MBC, n= 2 POC pts & n= 2 healthy donors).
Methods were validated in spike-in experiments using different cell lines spiked into blood of healthy donors

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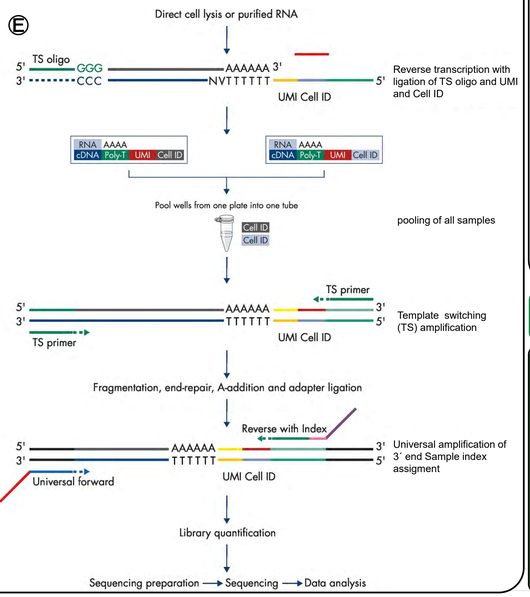

Fig. Ⓔ: The workflow of the QIAseq UPX 3' Transcriptome Kit
Applying the QIAseq UPX 3' Transcriptome Kit, each single cell was tagged with an individual cell ID-sequence and each RNA molecule was simultaneously tagged with an unique molecular identifier (UMI) during reverse transcription. Tagging of each individual cDNA molecule enabled pooling of all samples. The template switching amplification ensures the enrichment of only cDNA molecules containing UMIs for subsequent library preparation.
Amplified DNA was first fragmented, end repaired and A-tailed. During adapter ligation, the prepared DNA fragments were ligated at their 5' ends with a sequencing-platform-specific adapter. Subsequent library preparation steps were performed, followed by high-throughput NGS (Illumina platform) and Data analysis (QIAseq UPX 3' Transcriptome Analysis tool). (figure from QIAseq UPX 3' Transcriptome Handbook 02/2018, modified)

Results

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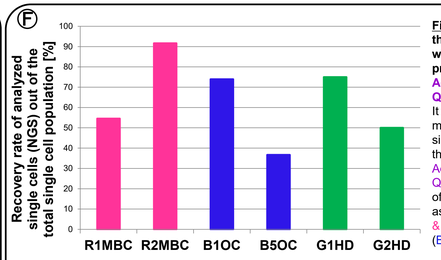

Fig. Ⓕ: Recovery rate of the single cells isolated with the QIAscout and processed with the AdnaTestSelect-QIAscout-UPX-workflow
It was possible to recover most of the isolated single cells picked with the QIAscout using the AdnaTestSelect-QIAscout-UPX-workflow of HDs (G1HD & G2HD) as well as MBC (R1MBC & R2MBC) and POC pts (B1OC & B5OC).

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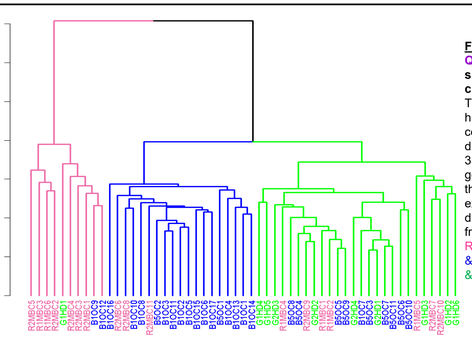

Fig. Ⓗ: AdnaTestSelect-QIAscout-UPX-workflow single cell RNAseq cluster analysis
The diagram shows the hierarchical clustering of correlated cases by differentiation using all 355 UPX confirmed genes. It could be shown that the UPX gene expression profiles differentiates single cells from MBC pts (R1MBC & R2MBC), POC pts (B1OC & B5OC) and HDs (G1HD & G2HD).

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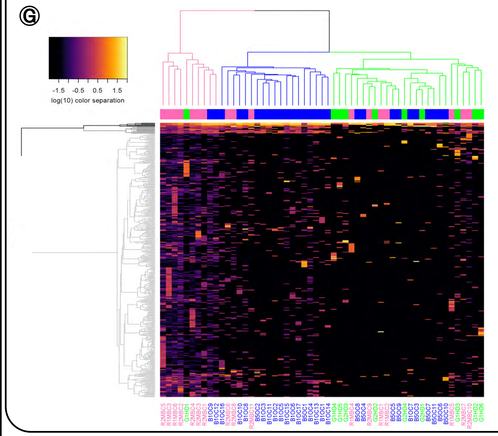

Fig. Ⓖ: Heatmap for case differentiation using all 355 genes that were positively confirmed by UMIs
The heatmap exhibits differential gene expression of the single cells from MBC pts (R1MBC & R2MBC), POC pts (B1OC & B5OC) and HDs (G1HD & G2HD).
(Cluster method: Wards, Distance: Euclidean, Transformation: $\log_{10}$)

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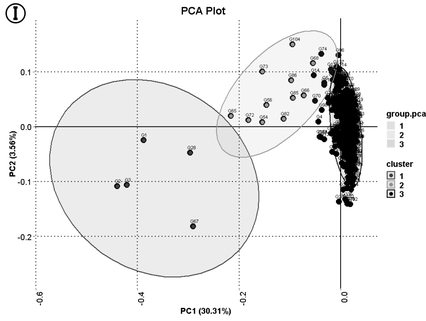

Fig. Ⓘ: Principal Component Analysis (PCA) Plot Selection
PCA (K-means grouping) identified genes of relevance for the case differentiation in Fig. Ⓗ. A group of 5 (dark grey) and 12 driver genes (grey) could be differentiated from the remaining genes (black) expressed in 55 single cells analyzed. These genes seem to be involved in epithelial to mesenchymal transition (EMT), resistance and cell movement.

Conclusion

The workflow evaluation with the QIAscout in CTC analysis shows:

- **the feasibility of the new AdnaTestSelect-QIAscout-UPX-workflow.**
- **that the QIAscout single cell isolation platform allows subsequent transcriptome analysis followed by NGS to get insights into single cell heterogeneity for further therapeutic strategies.**
- **that using the combination of AdnaTest*Select* & QIAscout shows an overall single cell recovery >50 %.**
- **both described workflows have to be further evaluated in more detail.**
- **a pending analysis of the comparison of these UPX results to traditional WTA.**

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