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A Novel Tissue Masking Technology Successfully Improves Tumor Cellularity for Clinical Next Generation Sequencing – Early Results of a Clinical Validation Study

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Background

- Clinical next generation sequencing (NGS) assays usually have a minimum tumor cellularity requirement.
- Standard macrodissection technique is sometimes not precise enough to enrich the tumor.
- We investigate the effectiveness of a novel tissue masking technology in improving tumor cellularity.

Design

- Archival NGS cases containing variants with a low variant allele frequency (VAF) of ≤10% and cases with no variants detected but with a low tumor cellularity of ≤20% were selected.
- Haematoxylin and eosin (H&E) stained sections were scanned into digital images and uploaded onto QuantumCyte's QCPRECISE!™ platform for annotation by a pathologist.
- A layer of chemical mask based on the annotated digital image is created and applied onto the tissue, leaving the tumor cells exposed (white regions) while masking the intervening stroma (black region) (Figure 1). DNA is extracted from the exposed tumor cells and sent for NGS.

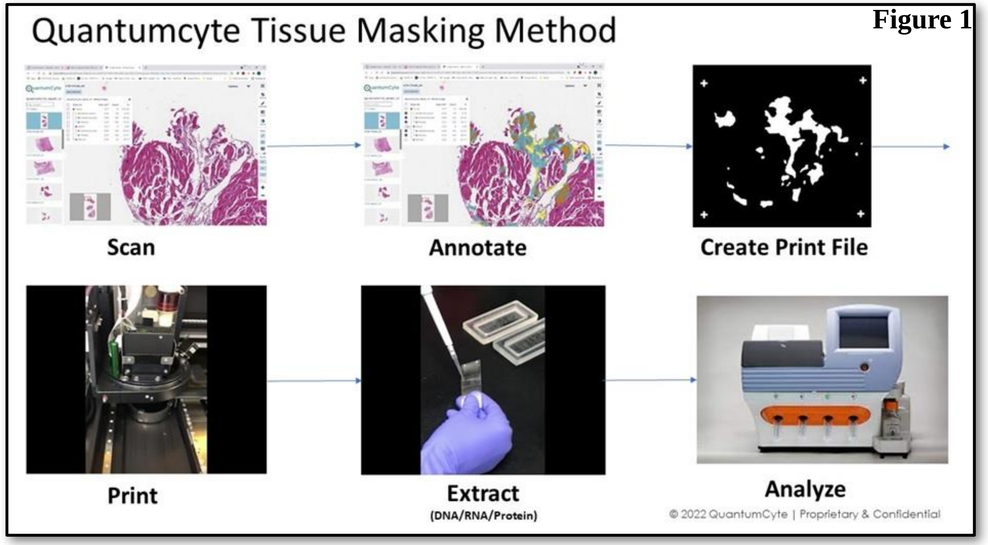

Figure 1

Results

Original NGS result	No. of cases (%)	Change in variant allele frequency (VAF) after QuantumCyte
Positive for variants	37 (88.1%)	+2.6% to +61.3% (mean 17%, median 11.3%)
	5 (11.9%)	-2.1% to -4.4%
	Subtotal n = 42 (100%)	
Negative for variants	8	1 case had new *TP53* and *APC* variants, 7 remained negative

- 49 cases with low VAF variants and 8 cases with no variants detected were included. Cases included a mixture of biopsies and resections and mostly lung and colorectal cancers.
- 50 of the 57 cases had successful masking and NGS. 7 cases with low VAF variants failed NGS after successful masking.
- 37 (88.1%) showed an increase in VAF compared to standard macrodissection. 5 cases also showed new variants in *APC*, *TP53* and *NF1*.
- Of the 8 cases with no variants detected in the original NGS run, 1 case had new variants detected in *TP53* and *APC* after using the masking procedure (Figure 2).

Diagnosis	NGS result	VAF 1	Tumour cellularity	Quantumcyte + NGS result	VAF 2	Largest change in VAF (VAF2 - VAF1)
Colon adenocarcinoma	No mutation detected in the panel	N.A.	20%	1. TP53 Exon5, c.524G>A / p.Arg175His (p.R175H) 2. APC Exon16, c.3960_3964delGAGCG / p.Glu1322fs (p.E1322fs)	60.90% 49.30%	60.90%

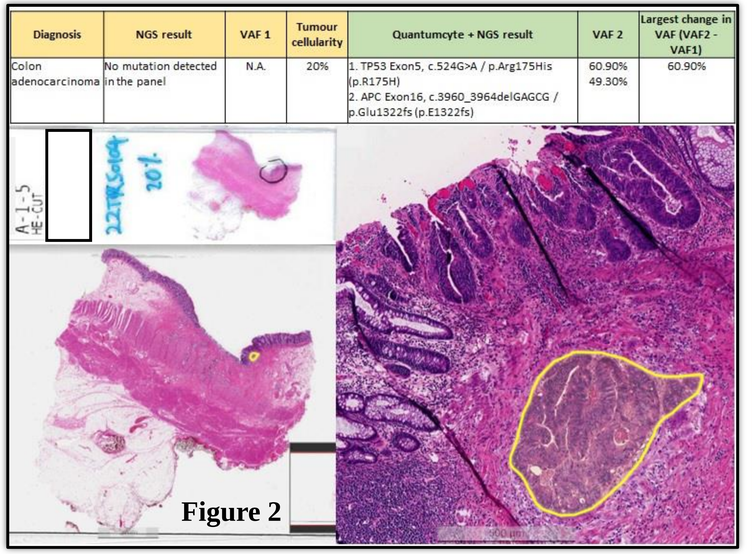

Figure 2

Conclusion

- QuantumCyte's novel tissue masking technology can enrich tumor specimens for clinical NGS.
- There is potential for this technology to salvage low tumor cellularity specimens that are unsuitable for clinical NGS.
- This may help patients avoid a repeat biopsy.
- Further studies will be required to fully evaluate its clinical utility, cost effectiveness and to investigate the small failure rate.