THE UTILITY OF PERIPHERAL BLOOD CIRCULATING TUMOUR CELLS FOR THE DETECTION OF *KRAS, EGFR* AND *BRAF* MUTATIONS IN PRIMARY LUNG CANCER

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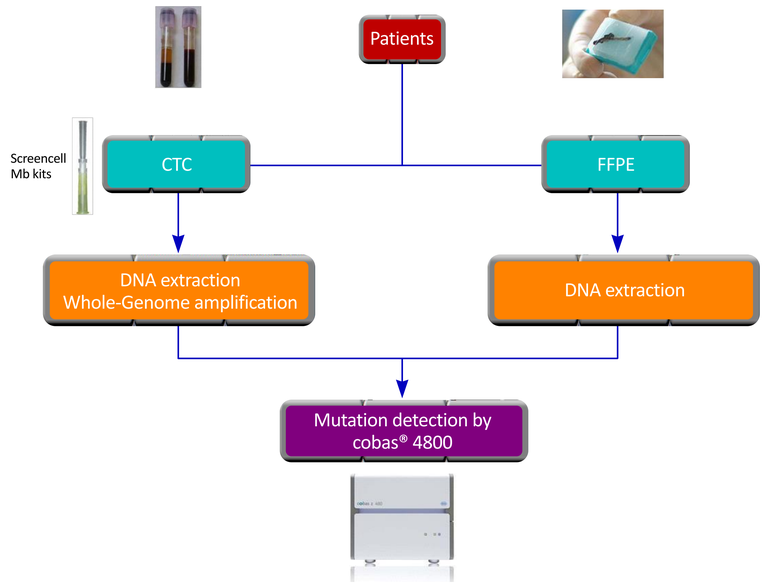
Background

Circulating tumour cells (CTCs) are present in the blood of a proportion of patients with lung cancer. However, it is currently unclear how suitable CTCs are for use in the detection of predictive genetic mutations. We sought to determine the utility of DNA extracted from CTCs to screen for the underlying primary tumour mutation.

Methods

Using ScreenCell™ MB devices, from 20/01/12 to 25/01/2013, CTCs were captured in peripheral blood of 100 patients who underwent surgery for lung cancer at The Royal Brompton Hospital. DNA was extracted using QIAamp DNA Micro kit (QIAGEN) followed by whole-genome amplification using GenomePlex® SingleCell WGA kit (Sigma). DNA from matched primary tumours was used as reference. Mutation detection in *EGFR* and *KRAS* genes was undertaken using cobas®4800 (Roche) and single-strand conformation analysis for *BRAF* gene. Sensitivity and specificity analyses were undertaken to measure predictive performance of mutation testing in CTCs.

Figure. The study pipeline



Results

The DNA extracted from CTCs, were of sufficient quality to allow mutation analyses to be successfully performed in 100%, 99%, and 98% of samples for *EGFR, KRAS,* and *BRAF* genes, respectively.

In CTC DNA, the *KRAS* mutation rate (codons 12/13 and 61) was 9.1% and concordance with the primary tumour was 78.8%. Six mutations were detected in CTCs, but not in primary tumours, and 13 mutations in primary tumours were not detected in corresponding CTC samples. Three mutations were detected in matched CTC and primary tumour specimens.

One mutation in *EGFR* was detected in CTC DNA and 3 mutations were detected in primary tumours. In all cases, the mutations were detected in discordant specimens. The concordance between mutations detection in CTCs and primary tumours was 95.8%.

BRAF V600E mutation was not detected in any sample.

In general, the results suggested low sensitivity but high specificity (Table). Due to low number of *EGFR* mutations detected, test performance results require further validation.

Table – The performance of the diagnosis of cancer using filter-based antibody-independent technique of CTCs trapping

Statistics	Gene	
	KRAS	*EGFR*
Sensitivity (95% CI), %	18.8 (4.05-45.6)	0 (0-70.8)
Specificity (95% CI), %	91.8 (83.0-96.9)	98.9 (94.1-100)
Positive predictive value (95% CI), %	33.3 (7.49-70.1)	0 (0-97.5)
Negative predictive value (95% CI), %	83.8 (73.8-91.1)	96.8 (91-99.3)

Conclusions

The result of our study indicates that the DNA extracted from CTCs can be used to screen for primary tumour mutations with reasonable concordance. Differences in the mutation results from the CTC and primary tumours needs to be explored in more detail and may be due to issues related to processing and / or tumour versus CTC heterogeneity.

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