Diagnostic Non-Invasive Biopsy Can Substitute Conventional Tissue Dependent Procedures in Suspected Cases of Lung Cancer

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Conflict of Interest:
Datar Cancer Genetics Limited offers commercial services in the domain of oncology.

BACKGROUND

- Histopathological evaluation (HPE) of tumor tissue obtained by invasive biopsy is routine procedure for confirmation of diagnosis in suspected cases of lung cancer,
- Invasive biopsies are associated with risks of Pneumothorax, Hemothorax, Empyema, Pulmonary Embolism, bleeding and pain,
- Non-invasive liquid biopsies are used to detect selected gene alterations in circulating cell-free tumor DNA (ctDNA) for targeted therapy selection,
- Wholesome, substitutive non-invasive biopsies for diagnosis are presently unavailable.

RATIONALE

- Circulating Tumor Associated Cells (C-TACs) are $EpCAM^{+}$ CK^{+}, $CD45^{-}$ cells of tumorigenic origin, in peripheral blood.
- Non-tumorigenic cells in peripheral blood have functional apoptotic mechanism, but C-TACs are resistant to apoptosis,
- An epigenetically acting stabilizing process can eliminate normal cells and confer survival privilege on apoptosis-resistant C-TACs,
- Sufficient C-TACs can be enriched and harvested for Immunocytochemistry (ICC) profiling with markers used in routine histopathological evaluations (HPE) of tumor tissue.

APPROACH

- 15 ml blood obtained from 498 known cases of lung cancer,
- C-TACs harvested from PBMCs by cell stabilization protocol,
- C-TACs identified by ICC with EpCAM, PanCK and CD45,
- ICC profiling of C-TACs with CK7, TTF1, Napsin, N-Cadherin, p40, Synaptophysin, Chromogranin A and Calretinin,
- Theranostic ICC profiling with PD-L1, ALK (D5F3).
- EGFR mutations, ALK, RET and ROS1 fusions in ctDNA by NGS.

STUDY POPULATION

Table 1. Age.

Status	Years
Minimum	21
Maximum	102
Median	60

Table 2. Gender.

Status	# (%)
Male	327 (65.7%)
Female	171 (34.3%)

Table 3. Metastatic status.

Status	# (%)
Metastatic	377 (75.7%)
Non-metastatic	35 (7.0%)
Unavailable	86 (17.3%)

Table 4. Therapy status.

Status	# (%)
Pre-treated	254 (51.0%)
Treatment Naive	163 (32.7%)
Unavailable	81 (16.3%)

Table 5. Radiological status.

Status	# (%)
Detectable Disease	428 (85.9%)
No Evidence of Disease	9 (1.8%)
Unavailable	61 (12.2%)

C-TACS YIELD AND CHARACTERIZATION

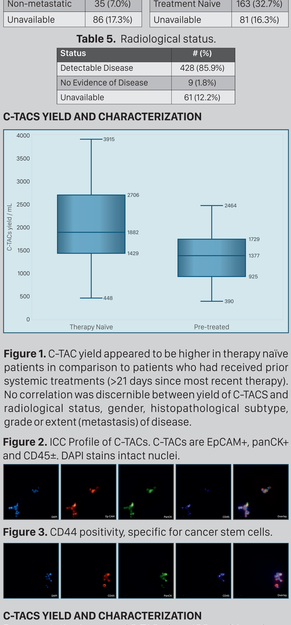


Figure 1. C-TAC yield appeared to be higher in therapy naïve patients in comparison to patients who had received prior systemic treatments (>21 days since most recent therapy). No correlation was discernible between yield of C-TACS and radiological status, gender, histopathological subtype, grade or extent (metastasis) of disease.

Figure 2. ICC Profile of C-TACs. C-TACs are EpCAM+, panCK+ and CD45±. DAPI stains intact nuclei.

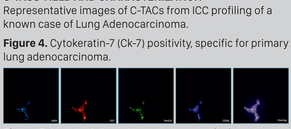
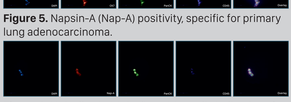


Figure 3. CD44 positivity, specific for cancer stem cells.



C-TACS YIELD AND CHARACTERIZATION

Representative images of C-TACs from ICC profiling of a known case of Lung Adenocarcinoma.

Figure 4. Cytokeratin-7 (Ck-7) positivity, specific for primary lung adenocarcinoma.

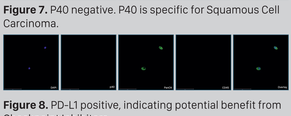


Figure 5. Napsin-A (Nap-A) positivity, specific for primary lung adenocarcinoma.

Figure 6. N-Cadherin (N-Cad) positive cells indicative of post Epithelial to Mesenchymal Transition.

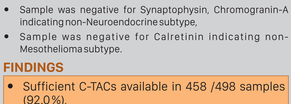


Figure 7. P40 negative. P40 is specific for Squamous Cell Carcinoma.

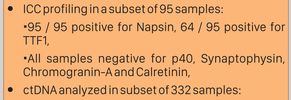
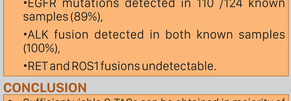


Figure 8. PD-L1 positive, indicating potential benefit from Checkpoint Inhibitors.



- Though, sample was positive for Napsin, it was negative for TTF-1,
- Sample was negative for Synaptophysin, Chromogranin-A indicating non-Neuroendocrine subtype,
- Sample was negative for Calretinin indicating non-Mesothelioma subtype.

FINDINGS

- Sufficient C-TACs available in 458 /498 samples (92.0%),
- ICC profiling in a subset of 95 samples:
  - 95 / 95 positive for Napsin, 64 / 95 positive for TTF1,
  - All samples negative for p40, Synaptophysin, Chromogranin-A and Calretinin,
- ctDNA analyzed in subset of 332 samples:
  - EGFR mutations detected in 110 /124 known samples (89%),
  - ALK fusion detected in both known samples (100%),
  - RET and ROS1 fusions undetectable.

CONCLUSION

- Sufficient viable C-TACs can be obtained in majority of samples indicating viability of approach for clinical application,
- ICC-characterization of C-TACs provides relevant diagnostic and theranostic information,
- C-TAC based non-invasive approach can substitute conventional procedures dependent on invasive biopsies,
- ctDNA based profiling of molecular alterations fulfills most clinical decision-making requirements in lung cancer.