Circulating Tumor Associated Cells in Esophageal Cancers are Resistance Educated per Previous Chemotherapy Treatments

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

BACKGROUND

- Innate and acquired chemoresistance common in Esophageal Squamous Cell Carcinomas (ESCC).
- Non-invasive real-time chemoresistance monitoring can guide precision therapy management
- Longitudinal chemoresistance profiling (CRP) of tumor derived cells (TDCs) is unviable since it necessitates repeated invasive biopsies.
- Conventional methods for harvesting Circulating Tumor Cells (CTCs) have low yields, due to which CRP of CTCs is not feasible.

RATIONALE

- Circulating Tumor Associated Cells (C-TACs) are EpCAM+, CK+, CD45± cells of tumorigenic origin, in peripheral blood.
- An epigenetically acting stabilizing process can eliminate apoptosis sensitive normal cells and confer survival privilege on apoptosis-resistant C-TACs.
- Sufficient C-TACs can be enriched and harvested for CRP

APPROACH

- 15 mL peripheral blood collected from 80 confirmed cases of ESCC, including 52 recently diagnosed and therapy naive and 28 pretreated.
- Viable C-ETACs enriched and harvested from PBMCs
- C-TACs treated with Standard of Care (SoC) agents for ESCC and apoptotic fraction estimated to determine resistance or sensitivity.
  - innate platin resistance in therapy-naive samples
  - acquired platin resistance in pre-treated patients.

DEMOGRAPHICS

Table 1. Age Distribution

	Therapy Naïve	Pre-treated
Minimum	16	30
Maximum	83	67
Median	63	61

Table 2. Gender Distribution

	Therapy Naïve	Pre-treated
Male	31 (59.6%)	22 (78.6%)
Female	21 (40.4%)	6 (21.4%)
Total	52	28

Table 3. Metastatic Status

	Therapy Naïve	Pre-treated
Metastatic	29 (55.8%)	25 (89.3%)
Non-Metastatic	12 (23.1%)	2 (7.1%)
Unknown	11 (21.2%)	1 (3.6%)

Table 4. Imaging Status

	Therapy Naïve	Pre-treated
Detectable	53 (100.0%)	23 (82.1%)
NED*	-	4 (14.3%)
Unknown	-	1 (3.6%)

*No Evidence of Disease

Table 5. Prior Lines of Therapy

	Therapy Naïve	Pre-treated
Minimum	-	1
Maximum	-	5
Median	-	2

C-TACS YIELD AND CHARACTERIZATION

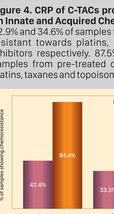


Figure 1. C-TACs Yield. The label-free enrichment process yielded sufficient C-TACs irrespective of treatment status.

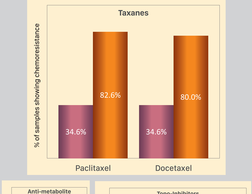


Figure 2. Characterization of C-TACs by Immunocytochemistry. Representative images of C-TACs from patients characterized by immunostaining. White bar at bottom left = 50 µm.

IN VITRO CHEMORESISTANCE PROFILING C-TACs

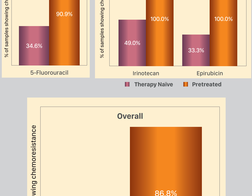
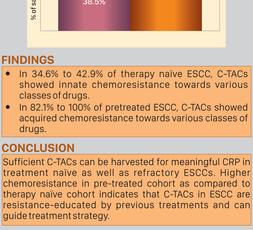


Figure 3. In vitro CRP. Representative images of C-TACs from a case of ESCC pre-stained with Calcein-AM and treated with Oxaliplatin (A-E), Cisplatin (F-J) and Carboplatin (K-O). After 12 h, % of apoptotic events cell death was determined by direct fluorescence-microscopic evaluation. Each row is a sequence of 5 images obtained at 0, 3, 6, 9 and 12 hours respectively. 80% cytotoxicity was observed in case of C-TACs treated with Oxaliplatin and Cisplatin, whereas 20% cell death was observed in C-TACs treated with Carboplatin. (P-T) are untreated cell control. White bar indicates 100 µm.

C-TACS ACCURATELY CONVEY CHEMO-ANTECEDENTS OF THE TUMOR

Figure 4. CRP of C-TACs provides real time information on Innate and Acquired Chemoresistance. 40.8%, 34.6% 42.9% and 34.6% of samples from therapy naive cases were resistant towards platins, taxanes and topoisomerase inhibitors respectively. 87.5%, 82.1%, 100% and 85.7% of samples from pre-treated cases were resistant towards platins, taxanes and topoisomerase inhibitors respectively.



FINDINGS

- In 34.6% to 42.9% of therapy naive ESCC, C-TACs showed innate chemoresistance towards various classes of drugs.
- In 82.1% to 100% of pretreated ESCC, C-TACs showed acquired chemoresistance towards various classes of drugs.

CONCLUSION

Sufficient C-TACs can be harvested for meaningful CRP in treatment naive as well as refractory ESCCs. Higher chemoresistance in pre-treated cohort as compared to therapy naive cohort indicates that C-TACs in ESCC are resistance-educated by previous treatments and can guide treatment strategy.