6684 Detection of PD-L1, HER2 and EGFR on circulating tumor cells in carcinoma patients

Jayant Khandare[1,2], Atul Bharde[1,2], Sreeja Jayant[1], Gourishankar Aland[1], Meghana Garbhe[1], Sayali Gosavi[1], Apoorva Janorkar[1], Purva Tikekar[1], Mrunmayi Patil[1], Vikas Jadhav[1], Ganesh Khutale[1], Amrut Ashturkar[1], Aravindan Vasudevan[1,2], Kumar Prabhash[4], Nirmal Raut[3], Pankaj Chaturvedi[4]

BACKGROUND

Small molecular inhibitors and immunotherapy have emerged as a novel alternative treatment regime for a variety of epithelial cancers.

A large number of clinical trials are in progress worldwide to gauge efficacy of tyrosine kinase inhibitors (TKI) or immune checkpoint inhibitors (ICI) against actionable targets such as receptor tyrosine kinases (RTKs) and program death ligand 1 (PD-L1).

Although highly effective, the outcome of PD-L1 based ICI or TKI against RTKs is vitally contingent on the presence of PD-L1 or RTK expression on cancer cells. Determining druggable targets on the basis of solid biopsy could be often misleading, especially if a patient has progressed in spite of chemotherapy.

PROBLEM STATEMENT

The intrinsic evolution of tumor cells along therapeutic selection pressure often contemplates alteration in expression of actionable molecular and immunological oncotargets. Therefore, biopsy-based information on molecular targets is inadequate for real-time treatment decisions. A multitude of quality compounds proper interpretation of immunohistological results. Additionally, serial biopsy is practically challenging for deep tissue tumors in case of progressive disease. This unmet need of reliable detection and real-time monitoring of actionable targets could be addressed by circulating tumor cells (CTCs).

We developed a CTC-based liquid biopsy assay to detect PD-L1, HER2 and EGFR expression in different epithelial cancers.

METHODS

We retrospectively evaluated peripheral blood samples from a total 134 carcinoma patients for the presence of CTCs expressing PD-L1, HER2 or EGFR markers respectively. Among these, 45 % patients had lung cancer, while 25 % and 20 % were presented with breast, GI and colorectal malignancies. Remaining were gall bladder, ovarian, prostate and head and neck cancer patients. All lung cancer patients were analysed for the presence of CTCs expressing PD-L1. CTCs were isolated from DCGI approved OncoDiscover technology based on immunomagnetic targeting of epithelial cell surface molecule (EpCAM) antibody. EpCAM targeted, magnetically isolated cell was considered as a CTC on the basis of expression of cytokeratins, absence of CD45, and prominent presence of a DAPI stained nucleus. Presence or absence of aforesaid markers was determined using automated fluorescence imaging. Expression of PD-L1, HER2 or EGFR was detected by fluorescence microscopy using fluorescently labelled anti PD-L1, HER2 or EGFR monoclonal antibodies respectively. Based on fluorescence intensity CTCs were binned as PD-L1, HER2 or EGFR negative for no detectable fluorescence signals, weakly or strongly positive, based on low or high fluorescence signals.

FIGURES

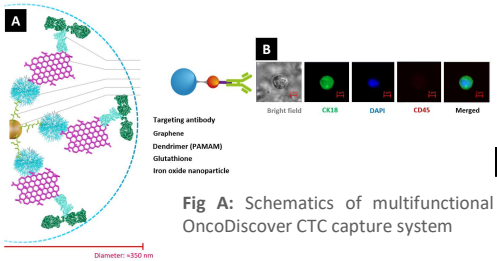

Fig A: Schematics of multifunctional OncoDiscover CTC capture system

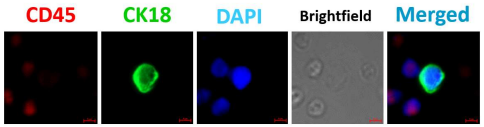

Fig B: CTCs captured from a breast cancer patient's blood using OncoDiscover Test

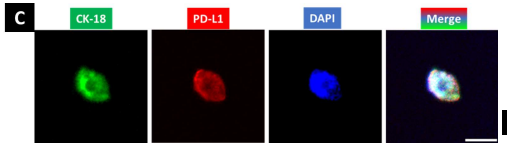

Fig C: PD-L1 expression on a CTC obtained from a lung cancer patient using OncoDiscover test.

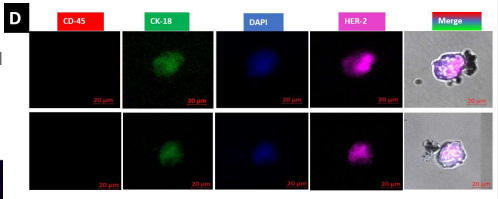

Fig D: HER2 expression on a CTCs obtained from a breast cancer patient OncoDiscover Test.

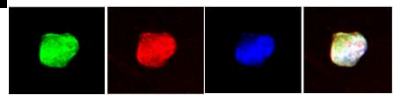

Fig E: OncoDiscover test detects EGFR expression on a CTC obtained from a lung cancer patient.

Table 1.

CTC Marker	Total no. of patient samples	Total no. of CTC positive patients	Total no. of CTC positive patients with PD-L1 expression
PD-L1	136	58.08 %	81 % positive for PD-L1

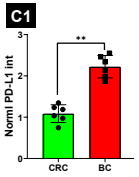

Fig C1: CTCs obtained from BC patients show higher relative PD-L1 expression compared to CTCs obtained from CRC patients.

Table 2.

No of tests	Mean CTCs	No of patients with HER-2 + ve expression	No of HER-2 expression in TNBC patients	HER-2 expression in HER-2 +ve patients	HER-2 expression in HER-2 -ve patients
				Concordance	Discordance
n=83	1.3	50% (n=41)	100% (n=9)	50%	90.91% (n=11)

CTCs with HER2 expression reveals real time status, compared to biopsy immunohistochemistry.

RESULTS

- Among evaluated cohort, 51% of all CTCs showed the presence of PD-L1 expression, while 63% showed HER2 positive CTCs (from breast cancer patients).
- 20% from the PD-L1 positive population showed strong PD-L1 expression. 78% of CTCs from lung cancer patients showed presence of detectable PD-L1 signal, while 66 % breast, GI and CRC patients showed CTCs with PD-L1 expression.
- CTCs from HNC and gall bladder cancer patients showed lower PD-L1 expression (25% and 50% respectively).
- Among CTCs originating from different cancer types, breast cancer CTCs showed higher mean expression of PD-L1 compared to CTCs from colorectal cancer patients.
- A clear subset of CTCs for PD-L1 and HER2 expression was observed in lung and breast cancer patients respectively, suggesting for the heterogeneity in expression or presence of different subclones within the same tumor type.
- Among all CTCs evaluated for EGFR expression, 50 % showed presence of detectable EGFR compared to the cut-off value (data not shown).

CONCLUSIONS

- CTCs isolated from cancers of epithelial origin showed the presence of PD-L1. Similarly, CTCs obtained from breast and lung cancer patients showed HER2 and EGFR expression, respectively.
- CTC can be used as a real-time surrogate for molecular profiling of PD-L1, HER2 and EGFR expression, besides being a prognostic marker.
- Detection of PD-L1, HER2 and EGFR in CTCs offers a potential and viable alternative for immunotherapy or targeted therapy decisions in a vast majority of epithelial cancers.
- CTC based biomarkers are dynamic biomarkers, over to tissue based static signatures. And useful when the tissue is inadequate and unavailable.

[1]Actorius Innovations and Research, Simi Valley, USA and Pune, India; [2]OneCell Diagnostics, Pune, India; [3]Bhaktivedanta Hospital and Research Institute, Mumbai, India;
[4]Tata Memorial Hospital, Mumbai, India
Correspondence: Dr. Jayant Khandare
jayant@actorius.co.in

www.OncoDiscover.com