Delineating immune networks in colorectal cancer to predict effects of immune checkpoint inhibitors using CANScript platform technology preserving tumor microenvironment

Nilesh Brijwani1, Nikita N Karandikar1, Vinod D Radhakrishna1, Muthusamy Oliyarasi1, Dency D Pinto1, Santhosh Kumar2, Sreeja Balakrishnan1, Bala Babu3, Manoj Rajappa1, Debapriya G Mehrotra1, Priyanka Chevur1, Archasubhra Ghosh1, Saravanan Thiyagarajan1, Biswanath Majumder1, Padhma Radhakrishnan1 and Pradip K Majumder1

1Mitra Biotech, Bangalore, India, and 2Grow Research Lab, Bangalore, India.

Abstract

Colorectal cancer (CRC) is one of the leading causes of cancer related mortality. Recent findings from clinical trials showed that immunomodulator drugs are making rapid inroads in the arena of clinical oncology. However, existing biomarkers and 3D platforms are not equipped to offer reliable prediction of response to these drugs in individual context. We recently engineered a personalized ex vivo systems biology based platform of patient tumors (Majumder B et al. Nat. Commun. 2015; Goldman A et al. Nat. Commun. 2015) and a companion in vivo patient derived immune reconstituted xenograft model (MI-HTX) preserving multiple critical phenotypic profiles orchestrating tumor-immune cross talk and dysfunction. We further performed baseline characterization of immune markers (CD4, CD8, CD68, CXCR4, CD45-RO). Intratumor heterogeneity of both M1 and M2 phenotypes were observed irrespective of clinico-pathological features. Baseline CD68 enrichment status was found to be correlated with therapy failure for some patients where clinical outcome was available. Flow cytometry and microarray analysis showed preserved expression of diverse sets of functional CD4, CD8 and NK phenotypes along with degradation markers (CD107a), IFNγ and FoxP3 in both in vivo and ex vivo tumors reconstituted with ex vivo primed PBMC. The comparative response profiling of these models following treatment with anti CTLA4 and PD1/L1 molecules suggested enhanced antitumor effect (as measured by visibly assayed, inhibition of K Ul-1 and concurrent induction of Caspase-3). Together, these findings highlight the strengths of immune-competent functional testing tools to stratify patients for precision immunotherapy where direct response prediction biomarkers are still elusive.

Results

We further performed baseline characterization of immune markers (CD4, CD8, CD68, CXCR4, CD45-RO). Intratumor heterogeneity of both M1 and M2 phenotypes were observed irrespective of clinico-pathological features. Baseline CD68 enrichment status was found to be correlated with therapy failure for some patients where clinical outcome was available. Flow cytometry and microarray analysis showed preserved expression of diverse sets of functional CD4, CD8 and NK phenotypes along with degradation markers (CD107a), IFNγ and FoxP3 in both in vivo and ex vivo tumors reconstituted with ex vivo primed PBMC. The comparative response profiling of these models following treatment with anti CTLA4 and PD1/L1 molecules suggested enhanced antitumor effect (as measured by visibly assayed, inhibition of K Ul-1 and concurrent induction of Caspase-3). Together, these findings highlight the strengths of immune-competent functional testing tools to stratify patients for precision immunotherapy where direct response prediction biomarkers are still elusive.

Overview of CANScript™

Clinical information

- CANScript platform and assays
- Cell viability
- Pathological and morphological analysis
- Cell proliferation and cell death
- Clinical history
- Tumor Stage
- Biopsy
- Pathology

Clinical Correlation

- D1 + D2
- D3 + D4
- CR
- NR

Results

Efficacy of anti CTLA4 in modulating immune-check point phenotypes and efficacy prediction in CANScript™ CRC tumors were cultured ex vivo for 72 hours in presence or absence of CTLA4 inhibitor. A: Activation of cytotoxic immune markers were measured by IFNγ. B: M score generated based on functional and immune modulation were integrated to predict efficacy against individual tumors.

Summary

- Tumor heterogeneity might be one of the causes why two patients with similar genetic background responses differently. CANScript™ technology mimics patient tumor heterogeneity and tumor immune-microenvironment.

- We have also observed tumor infiltrating immune cells heterogeneity in patients with colorectal cancer. Data showed differential cytokines and chemokines expression in different patient tumors.

- Number of infiltrating immune cells (like CD8, CD68 CD45RO) are not only varied in different patients but their localization is also heterogeneous.

- Key M1 and M2 immune cells also differ in chemo sensitive and refractory patient tumors.