Temporospatial heterogeneity in metastatic colorectal cancer (mCRC)

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Background:

- Anti-epidermal growth factor receptor (EGFR) therapy has the potential to increase antitumor immune responses. Thus, therapeutic strategies targeting EGFR and immune checkpoints may benefit patients (pts) with mCRC.
- We conducted a phase Ib/II study of cetuximab (C) with pembrolizumab (P) in patients with RAS wild-type (RASwt) mCRC.
- C+P increased intratumoral cytotoxic T-cells (CTL) and natural killer (NK) cells (Boland et al, ASCO 2020) but had modest antitumor efficacy (Fountzilas et al, ESMO GI 2020).
- Here, we present updated baseline (pre-Tx) and on-treatment (post-Tx) tumor tissue analysis.

Methods:

- Study Schema (**Figure 1**). 44 pts with mCRC enrolled:
  - 14 pts with matched Pre- and Post-Tx Bx.
  - 24 pts with Pre-Tx Bx only, 1 with Post-Tx Bx only.
- Multiplex immunohistochemistry (IHC) was performed for PD-L1, other T-cell exhaustion (TIM3, CTLA4, LAG3) and activation (OX40) markers (**Figures 2 and 3**).
- T-cells were characterized as:
  - Partially Activated-1 (OX40+/PD-L1-, 1/3 exhaustion markers+)
  - Partially Activated-2 (OX40+/PD-L1-, 2/3 exhaustion markers+)
  - Partially Activated-3 (OX40+/PD-L1-, 3/3 exhaustion markers+)
  - Exhausted-1 (OX40-/PD-L1-, 1/3 exhaustion markers+)
  - Exhausted-2 (OX40-/PD-L1-, 2/3 exhaustion markers+)
  - Exhausted-3 (OX40-/PD-L1-, 3/3 exhaustion markers+)

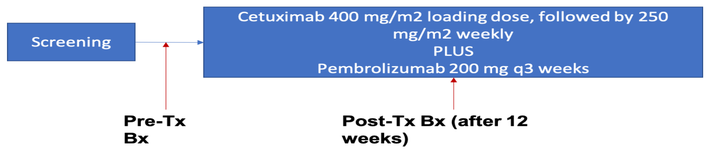


Figure 1: Study Schema

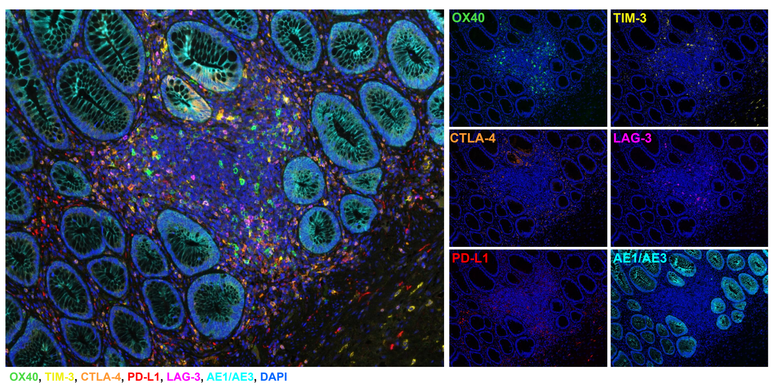


Figure 2: Representative multiplexed images with the composite image (left panel) and individual markers (right panels).

Results:

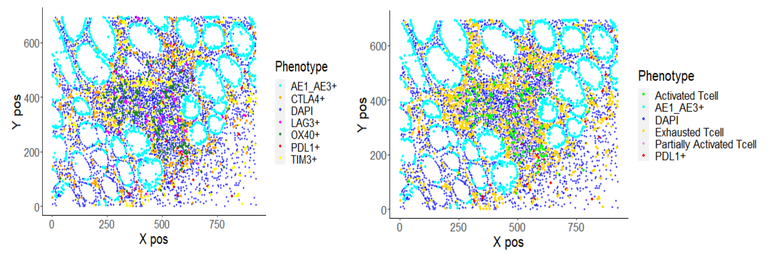


Figure 3: Phenotyping of the individual tumor and Bx specimens was performed using InFORM software to speciate different immune subsets within the tumor microenvironment.

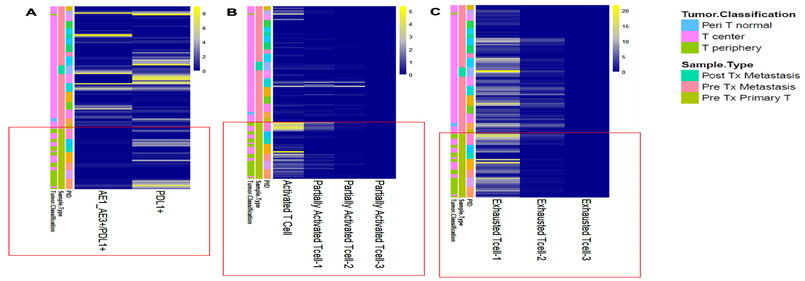


Figure 4: Heatmap classified based on Pre-Tx Primary Tumor (red box) vs. Pre-Tx Metastasis Bx showing number of (**A**) PD-L1 positive tumor cells (AE1_AE3+/PDL1+) and PD-L1 positive cells in tumor microenvironment (AE1_AE3-/PDL1+); (**B**) activated T-cells subset; (**C**) and exhausted T-cells subsets.

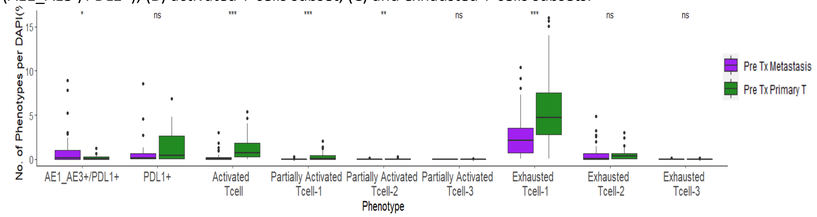


Figure 5: Quantitation of signals per specimen. There are more PDL1 positive tumor cell in the Pre-Tx Metastatic Bx. While there are more activated, partially activated T cell-1 and exhausted T cell-1 in the primary tumor specimens (*p<0.05, ***p<0.001)

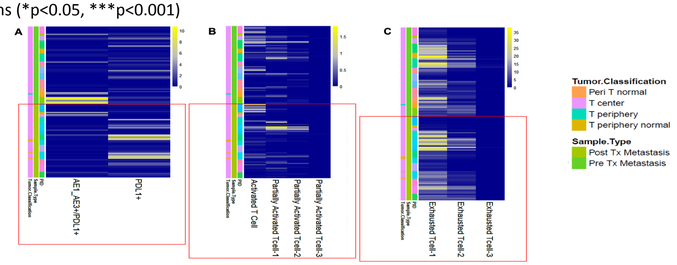
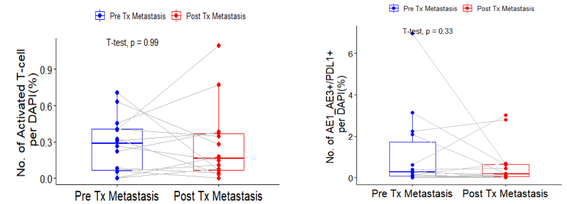


Figure 6: Heatmap classified based on Pre-Tx vs. Post-Tx (red box) Bx showing number of (**A**) PD-L1 positive tumor cells (AE1_AE3+/PDL1+) and PD-L1 positive cells in tumor microenvironment (AE1_AE3-/PDL1+); (**B**) activated T-cells; (**C**) exhausted T-cells.

Conclusions:

- The tumor microenvironment (TME) in mCRC is characterized by high numbers of cells with expression of at least one marker of exhaustion other than PD-L1.
- Expression of PD-L1 is higher in metastatic sites vs. primary tumor.
- The number of activated T-cells is higher in primary tumor vs. metastatic sites.
- Treatment with C+P increased the number of activated and partially activated-1 T-cells and decreased PD-L1+ tumor but there was no change in the number of PD-L1- tumor cells expressing other exhaustion markers.
- Persistence of exhausted CTLA4+, and or TIM3+, and or LAG3+ T-cells may explain the lack of additive benefit from P.
- Combinations with agents targeting other T-cell exhaustion pathways may be necessary to improve the antitumor efficacy of the EGFR- plus anti-PD1-based regimens.
- Evaluating the primary tumor may not be reflective of the immunosuppression in metastatic sites.

Preliminary Intra-Patient Changes:



Ongoing investigation is determining if select treatment associated changes in T-cell populations (e.g. increase in activated T-cells) is associated with exceptional responses within this study cohort.

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