Characterizing immunotherapy-induced lymphocyte infiltration at the single patient level using CANscript™, an ex-vivo human tumor model

Munisha Smalley1, Basavaraja U Shanthappa1, Hans Gertje1, Mark Lawson1, Baranedharan Ulaganathan1, Allen Thayakumar1, Laura Maciejko1, D.C. Doval1, Anurag Mehta1, Padma Radhakrishnan1, Pradip Majumder1, Aaron Goldman1

1. Integrative Immuno-Oncology Center, Mitra Biotech, Woburn, MA 01801, USA. 2. Rajiv Gandhi Cancer Institute, New Delhi, India.

Background: The presence and activity of lymphocytes within the tumor is critical for clinical response to cancer immunotherapy, such as immune checkpoint blockade. Tumors with poor T-cell infiltrated phenotypes, often referred to as a "cold" tumor, is associated with modest clinical response. High baseline expression of effectors (CD8+ T cells) and lymphocytes is considered "hot" and patients are predicted to respond more favorably to treatment. However, patient-to-patient response and durability remains highly variable. There is an urgent gap in available methods to study lymphocyte infiltration, trafficking and spatial heterogeneity induced by different cancer immunotherapeutics in individual patients. Moreover, there is a poor correlation between therapy-induced lymphocyte infiltration with clinical response, which could be shaped using personalized approaches to therapy.

Methods: Here, we used CANscript™ an ex-vivo human tumor model that recapitulates and preserves the native, patient-autologous tumor microenvironment, including autologous patient-derived peripheral blood mononuclear cells (PBMC). Utilizing fresh from breast cancer patients classified as either "cold" (N=5) or "hot" (N=5), we studied lymphocyte infiltration under pressure of anti-PD-1 immune checkpoint blockade ( Pembrolizumab) over a 72h time course compared to tumor stroma (Ts) and control. Using fluorescent labelling and flow cytometric analysis we characterized infiltrating lymphocytes, studying the different T-cell sub-population effects in PBMCs and lymphocytes and immunochemistry responses with spatial analyses with multiplex immunohistochemistry (CD3, CD4, CD8) to map spatial heterogeneity of tumor cells and lymphocytes before and after treatment, ex-vivo.

Results: We determined that immune checkpoint blockade induced unique patterns of migration and infiltration of effector T-cells (Tep) and T-regulatory cells (Treg) in 'hot vs' 'cold' tumors. Furthermore, we determined that, in some tumors, "cold" cells can be driven towards a 'hot' phenotype characterized by trafficking of active immune lymphocytes following treatment, which corresponded to differential ratio of Tep to Treg compared to baseline.

Concluding remarks: Taken together, these data demonstrate the utility of CANscript™ as a platform to characterize response to immunotherapy in a spatial context, providing insight into the migratory patterns of immune cell subsets at the individual patient level. Such an advance in our predictive model for immuno-modulators may help guide treatment decisions for clinicians while simultaneously functioning as a platform to study and discover mechanisms of clinical efficacy for emerging drug combinations.

Model Development - Lymphocyte Infiltration

1. Live tissue from breast cancer patient
2. Therapy interrogation: ex-vivo/labeled lymphocytes in culture
3. Studying dynamic T-cell infiltration
4. Multiplex IHC for spatial heterogeneity

Fig. 2 Characterizing response to anti-PD-1 therapy in multiple tumor types: A) Patient demographic and tumor (subtypes B) M-Score across multiple patient samples tested in CANscript™ using Pembrolizumab. C) Pair-wise expression of tumor infiltrated lymphocytes following vehicle (IgG) or Pembrol treatment (72h). D) Single patient (Breast EFF-HER2; all immune (suspended) and dissociated) representative VSNIE plots, indicating differential Treg population following vehicle (IgG) and Pembrol treatment in CANscript™. Plot colored on CD3+ population.

Fig. 3 Studying therapy-induced lymphocyte infiltration in a spatio-temporal context using CANscript™. 1. Fresh tumor containing stroma is harvested along with patient-autologous peripheral blood lymphocytes, which have been pre-labeled. 2) CANscript™ is performed using the co-culture of tumor/stroma biopsy with pre-labeled lymphocytes. 3) Tumor is harvested at 24h, 48h, and 72h post-treatment, and labeled for multi-colored flow cytometric analyses. Intratumoral TILs and stromal T cells are captured. 4) Tumor tissue is fixed and paraffin-embedded. T-cells representative expression patterns are interrogated and qualitatively assessed using fluorescent multiplex IHC.

Fig. 4Non-uniform, dynamic lymphocyte infiltration in patient-specific manner. A) Immunofluorescence expression of two representative breast cancer patient tumor tissues profiled for their CD4+CD8+ expression following 72h in CANscript treated with vehicle (IgG) or Pembrol. Note the change from "hot" to "cold" lymphocyte subsets before and after treatment. B) (IgG)-treated CANscript was assessed for spontaneous infiltration of CD4+ lymphocytes at 24h intervals at the patient-specific level. C) Viability of all lymphocytes (infiltrated + intrinsic TIL) vehicle (IgG) or Pembrol-treated CANscript. D) Total CD4+ lymphocyte infiltration in vehicle and Pembrol-treated tumor tissues at 24, 48h, and 72h.

Fig. 5 CANscript™ captures non-uniform infiltration of distinct immune subsets under vehicle control or drug-induced conditions. Pre-labeled T-cell and immune subsets were assessed using flow cytometry from vehicle and Pembrol-treated breast CANscript, post-co-culture with PBMCs, at 24, 48h, and 72h to profile infiltrating immune subsets (A) Treg cells identified by CD4+CD25+CD127L37+ (B) cytokotic CD8+CD57 T-cells and (C) CD69+ Natural Killer cells were analyzed for viability.

Fig. 6 Multiplexed IHC captures spatial heterogeneity of tumor infiltrating lymphocytes in patient-matched parallel and embedded tumor slices derived from breast tumor tissues, vehicle (IgG) and Pembrol-treated at T24, stained for Pannexin1 (magenta), CD3 (green), CD4 (yellow), CD8 (white), stained with DAPI (blue) and all of overlay markers. Imaging used PerkinElmer Vectra Polaris, which enables downstream quantitative pathology.

Conclusions: Dynamic, spontaneous infiltration of lymphocytes into the tumor (aka TL) is a key factor for immune-based tumor rejection. However, little is known about the role of immunotherapy or other anti-cancer agents as they modulate TLs. CANscript™ enables a platform to study the antitumor effect of different therapies along with stochastic lymphocyte infiltration at the individual patient-level. By integrating our algorithm-driven predictive clinical assessment (M-Score) we are able to provide unique understanding towards the role of dynamic TL with clinical response to therapy. Our data demonstrate that high-interpatient variability in immune modulation, and response to immunomodulators, suggests high importance of integrating a multi-dimensional approach to personalized cancer medicine.