Transcriptomic Profiling Identifies Immunotherapy-Responsive Phenotypes in Microsatellite-Stable Metastatic Colorectal Cancer

Poster C-T.35

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Background

- Colorectal cancer (CRC) is the second leading cause of cancer-related deaths globally, with rising incidence among younger adults[1]
- While immunotherapy has transformed outcomes in many cancers, the ~95% of metastatic CRCs (mCRC) that are microsatellite-stable (MSS) remain largely refractory to immune checkpoint inhibitors (ICIs)[2,3]
- Botensilimab (BOT) is a multifunctional Fc-enhanced anti–CTLA-4 with novel FcγR-dependent mechanisms to enhance T-cell priming, reduce intratumoral Tregs, and activate antigen-presenting cells[4]
- BOT ± balstilimab (BAL; anti–PD-1 pharmacologically comparable to approved PD-1 inhibitors) has shown activity in "cold" and ICI-refractory tumors, including heavily pretreated patients with MSS mCRC and no active liver metastases (NLM)[5-9]
  - In MSS mCRC NLM, BOT+BAL demonstrated an objective response rate (ORR) of 21%, disease control rate (DCR) of 69%, and 3-year overall survival (OS) rate of 33% (phase 1b C-800-01 trial)[9]
- However, clinical benefit with BOT+BAL remains heterogeneous, and the molecular and tumor microenvironment (TME) determinants underlying response and resistance are poorly understood
- **Hypothesis**: We hypothesized that distinct transcriptomic and TME states underlie the heterogeneous responses to BOT±BAL in MSS mCRC, and that treatment actively remodels tumors toward more immune-enriched phenotypes
- **Objective:** Define immunotherapy-responsive molecular and TME states in MSS mCRC by profiling pre- and on-treatment biopsies from BOT±BAL–treated patients, integrating bulk RNA-seq analyzed using self-organizing map (SOM) machine learning with cellular deconvolution and TME ecotyping, and correlating these states with clinical outcomes

Methods

Data Collection

- Tumor samples were collected from 49 patients with advanced refractory MSS mCRC enrolled in the phase 1b C-800-01 trial of BOT±BAL (NCT03860272; data cut-off date: March 13, 2025)
- Bulk RNA-seq (Personalis ImmunoID NeXT) was performed on 65 tumor samples:
  - 63 metastatic biopsies (liver, lung, abdomen, lymphatic system, pelvis)
  - 2 primary colorectal tumors
- A subset of biopsies (N=16) comprised paired samples, collected before (PRE) and during (ON) treatment
- Clinical benefit was defined by best overall RECIST v1.1 response (PR/SD/PD) and best percentage tumor change from baseline; SD and PD were subdivided by presence or absence of active liver metastases

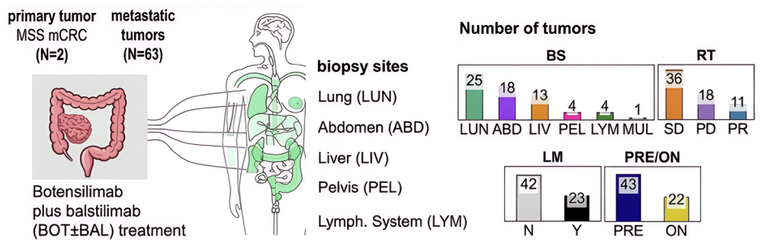

Figure 1. Study Cohort and Tumor Sample Collection

SOM Analyses

- **SOM analysis** (oposSOM v3.0)[10]: 17,250 genes were reduced to 1,600 metagenes and portrayed on a 40×40 SOM landscape. 28 D-modules were detected, of which 4 core modules formed the minimal signature for sample stratification
- **Stratification:** Unsupervised sample-similarity and silhouette analysis of SOM-transformed profiles defined the molecular types (MTs)
- **Deconvolution & ecotyping:** CIBERSORTx (cell-type fractions), EcoTyper (multicellular communities), and ESTIMATE (immune/stromal scores)
- **External classifiers:** pan-TME (Bagaev), pan-metastasis classification (PMC), Consensus Molecular Subtypes (CMS), and a constitutive 7-gene interferon immunophenotype score were projected onto the cohort
- **Statistics:** Group associations were tested by Fisher's exact and Kruskal-Wallis tests; response enrichment by binomial test; OS by Kaplan-Meier estimation with log-rank tests. Metagene-outcome associations were expressed as hazard ratios (HR) and odds ratios (OR)

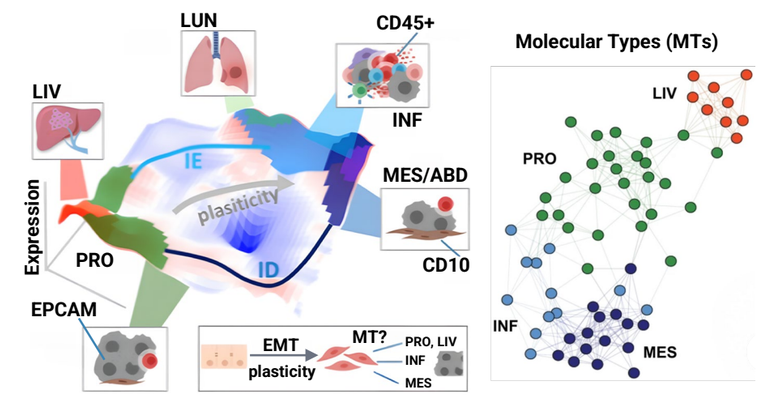

Figure 2. Molecular Type Identification with SOM Analysis

Results

Molecular Characterization of MSS mCRC

SOM Transcriptomic Mapping Defines 4 MSS mCRC MTs Along an Immunophenotype Axis

- The SOM portraits reveal the defining transcriptional signature of each MT:
- **Liver-Like (LIV):** marked by bile salt, lipid, and fatty-acid metabolism programs and consists largely of liver metastases with distinct metabolic properties
- **Proliferative (PRO):** displays mitotic-phase and proliferative stem-cell signatures, with an abundance of epithelial/tumor cells
- **Inflammatory (INF):** defined by immune-cell, inflammatory, and cytokine-related signatures reflecting an expanded immune TME compartment
- **Mesenchymal (MES):** shows membrane, synapse-related, and stromal signatures, corresponding to an expanded stromal compartment

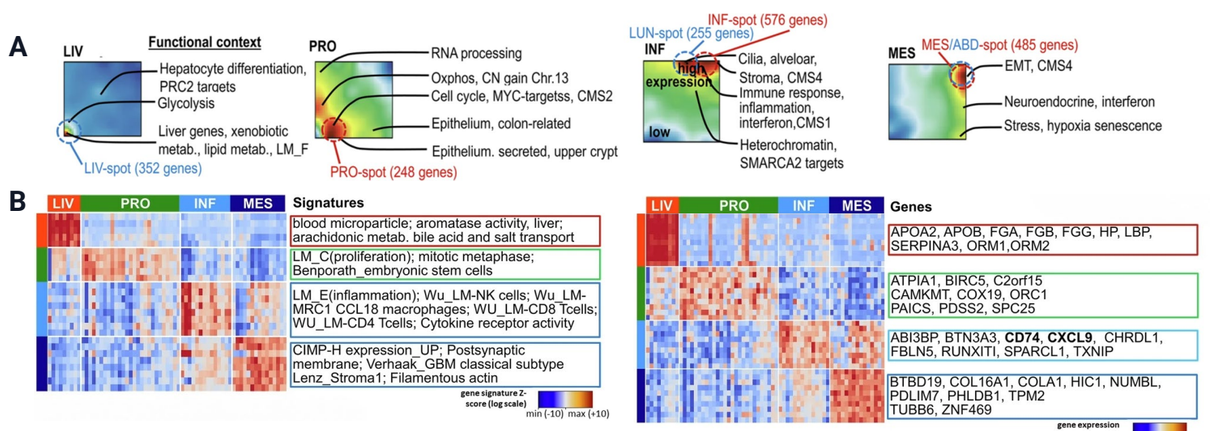

Figure 3. Representative SOM Portraits (A) and Transcriptomic and Gene Signatures (B) by MT

An Interferon/APOBEC3 Axis, not TMB, Marks Immunogenic Tumors

- An interferon score based on 7 gene expression signatures of CRC tumors[11] was used to group tumors by immunogenicity
- Differential gene expression analysis between the interferon-high and -low clusters revealed that expression of *APOBEC3-C/D/F/G/H* genes correlated with immunogenicity (correlation coefficient 0.55–0.87, $p < 1 \times 10^{-6}$)
- Interferon score and *APOBEC3* expression were the highest in the INF SOM module
- Immunogenicity was not captured by conventional measures of TMB

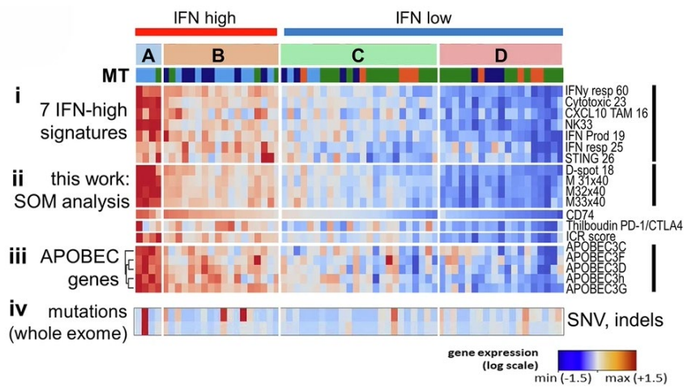

Figure 4. Interferon Signatures, *APOBEC3* Expression, and TMB

Clinical Outcomes by MT and TME Remodeling Following BOT±BAL

Immune-Enriched INF/MES States Associate With Deeper Tumor Regression and Longer Survival

- Tumors of the INF and MES types (N=29) showed significantly longer OS than LIV and PRO tumors (p=0.04, N=36). Concordantly, mapping OS onto the SOM landscape showed that expression in the INF and MES modules was associated with longer survival (HR < 1), whereas expression in the LIV and PRO modules was associated with shorter survival (HR > 1), relative to mean OS ($p < 0.05$)
- However, responses to BOT±BAL were not exclusive to INF/MES: a subset of PRO-classified tumors (N=3) also responded, including one patient with a particularly deep response
- BOT±BAL demonstrated activity across a spectrum of MSS tumor states

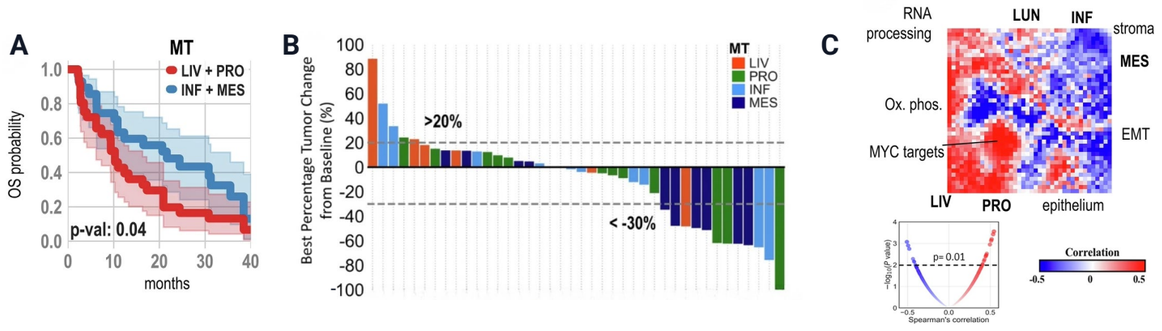

Figure 5. Clinical Outcomes by MT: Overall Survival (A), Best Tumor Response (B), and SOM Landscape Mapping (C)

BOT±BAL Shifts Tumors Toward Immune-Enriched States

- Comparing the deconvoluted cellular composition of paired pre- and on-treatment biopsies revealed infiltration of $CD8^+$ and $CD4^+$ T cells, anti-tumoral M1 macrophages, NK cells, and follicular helper T cells into the TME, alongside depletion of epithelial tumor cells and naïve macrophages
- Differential gene expression analysis identified upregulation of several immune-related genes, including *ZNF683* (a marker of non-exhausted T cells predictive of anti–PD-1 response) as well as *CXCL9/10*, *IFN-γ*, *GZMA/B/H*, *CCL5*, and *APOBEC3-G/H*
- Pathway analysis showed that upregulated genes were enriched for interleukin production, chemokine and interferon signaling, and NK activation, whereas downregulated genes reflected epithelial, developmental, and stress-state programs

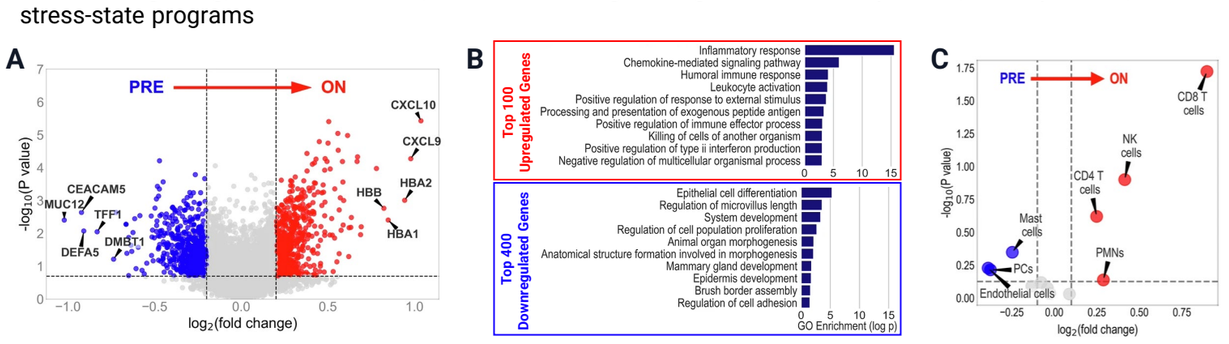

Figure 6. Differential Gene Expression (A), Pathway Enrichment (B), and Differential Cell Type Abundance (C) Following BOT±BAL

Conclusions

- SOM transcriptomic mapping identified **4 MSS mCRC molecular types** (LIV, PRO, INF, and MES) that reflect dominant TME programs along an immunophenotype axis rather than rigid categories
- An **interferon-high/*APOBEC3*-high immunophenotype**, marked by *CD74* and *APOBEC3-C/D/F/G/H*, distinguished immunogenic INF/MES tumors despite low global TMB, supporting a hypothesis that focal mutational processes or neoantigen quality may contribute to immune recognition
- Within the largely immune-cold MSS mCRC setting, relatively more **inflamed/interferon-high TME** states were associated with **deeper tumor regression and longer OS after BOT±BAL**; however, responses were not restricted to INF/MES tumors, supporting BOT±BAL activity across a spectrum of MSS tumor states
- **BOT±BAL remodels the TME toward a more immunogenic state**, with increased CD4/CD8 T cells, NK cells, M1 macrophages, interferon/chemokine signaling, and cytotoxic effector programs
- These findings support **transcriptomic and TME-state profiling** as a **potential biomarker strategy** to identify patients with MSS mCRC most likely to benefit from next-generation ICI therapy, and to guide rational combinations for immune-cold tumors

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Acknowledgments: The research was supported by the Higher Education and Science Committee of the Republic of Armenia (grant number 25RL-1F024).

References: 1. Morgan E, et al. *Gut*. 2023;72:338–344. **2.** Buchler T. *Front Oncol*. 2022;12:888181. **3.** Guven DC, et al. *Oncologist*. 2024;29(5):e580–e600. **4.** Chand D, et al. *Cancer Discov*. 2024;14(12):2407–2429. **5.** O'Malley DM, et al. *Gynecol Oncol*. 2021;163(2):274–280. **6.** O'Malley DM, et al. *J Clin Oncol*. 2022;40(7):762–771. **7.** Gordon MS, et al. Presented at: ESMO 2025. Presentation #1517MO. Berlin, Germany. **8.** Atkins MB, et al. Presented at: ASCO 2026. Poster #259. Chicago, IL, USA. **9.** Schlechter BL, et al. Presented at: ESMO GI 2026. Poster #91P. Munich, Germany. **10.** Wirth H, et al. *BMC Bioinformatics*. 2011;12:306. **11.** Acha-Sagredo A, et al. *Cancer Cell*. 2025;43:292–307.e7.

Abbreviations: ABD, abdomen; BAL, balstilimab; BOT, botensilimab; BS, biopsy site; CD, cluster of differentiation; CMS, consensus molecular subtype; CRC, colorectal cancer; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DCR, disease control rate; EMT, epithelial-to-mesenchymal transition; Fc, fragment crystallizable; FcγR, Fc gamma receptor; GO, Gene Ontology; HR, hazard ratio; ICI, immune checkpoint inhibitor; ID, immune depleted; IE, immune enriched; IFN, interferon; INF, inflammatory molecular type; LIV, liver-like molecular type; LM, liver metastasis; LUN, lung; mCRC, metastatic colorectal cancer; LYM, lymphatic system; MES, mesenchymal molecular type; MUL, multiple sites; MSS, microsatellite stable; MT, molecular type; NK, natural killer; NLM, no active liver metastases; ON, on-treatment; OR, odds ratio; ORR, objective response rate; OS, overall survival; PC, plasma cell; PD, progressive disease; PD-1, programmed cell death protein 1; PEL, pelvis; PMC, pan-metastasis classification; PMN, polymorphonuclear neutrophil; PRE, pre-treatment; PRO, proliferative molecular type; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; RNA-seq, RNA sequencing; RT, response type; SD, stable disease; SOM, self-organizing map; TMB, tumor mutational burden; TME, tumor microenvironment; Treg, regulatory T cell.

Poster presented at the European Conference on Computational Biology: August 31–September 4, 2026. Geneva, Switzerland.