colorectal cancer patients are non-responsive and the 5-year survival rate for advanced disease is <20%. Immunotherapeutic response has been associated with select members of the microbiome in melanoma; however, the potential benefit in colorectal cancer and the underlying mechanisms remain unclear. We sought to determine how specific members of the intestinal microbiome affect anti-tumor immunity in colorectal cancer (CRC) in hopes of discovering novel treatments and revealing potential hurdles to current therapeutic response in CRC patients.

Methods We utilized a carcinogen-induced mouse model of CRC and colonized half of the tumor-bearing mice with Helicobacter hepaticus (Hhep) 7 weeks post AOM. Tumor number was assessed 12 weeks post AOM. We isolated lymphocytes from the lamina propria, colonic epithelium, mesenteric lymph nodes, and tumor(s) to track the spatial and transcriptional Hhep-specific and endogenous immune responses during tumor progression through 5' single cell RNAseq, flow cytometry, and immunofluorescence. In addition, we utilized 16S sequencing and FISH to track Hhep colonization, location within the colon, and its impact on the surrounding microbiome.

Results We have found that rational modification of the microbiome of colon tumor-bearing mice through addition of a single bacteria, Hhep, led to tumor control or clearance and a significant survival advantage. Colonization led to the expansion of the lymphatic network and development of numerous peri- or intra-tumoral tertiary lymphoid structures (TLS) composed of Hhep-specific CD4 T follicular helper cells (TFH) as well as the bacteria itself. This led to an overall 'heating' of the tumor, wherein we saw an increase of CD4 T cell infiltration to the tumor core as well as an increase in CD103+ type 1 DC (cDC1) recruitment through increased chemokines such as CCL5 and XCL1. Hhep-specific TFH were both necessary and sufficient to drive TLS formation, increased immune invasion, and anti-tumor immunity.

Conclusions We have shown that addition of a single bacteria, Hhep, leads to a reduction in CRC tumor burden or clearance through lymphatic expansion, TLS formation, and remodeling of the tumor microenvironment, and that Hhep-specific T cells are required for tumor control. These studies suggest that rational modification of the microbiome and microbiome-specific T cells can positively impact anti-tumor immunity and may represent a unique immunotherapeutic target to turn resistant tumors into responsive tumors.

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679 HIGH FIBER DIET MODIFIES GUT MICROBIOME, PROPIONATE PRODUCTION, INTRATUMOR IMMUNE RESPONSE AND IS ASSOCIATED WITH OUTCOME IN PATIENTS WITH LUNG CANCER TREATED WITH IMMUNE CHECKPOINT INHIBITORS

Corentin Richard*, Myriam Benlaifaoui, Omar El Ouarzadi, Khoudia Diop, Antoine Desilets, Julie Malo, Wiam Belkaid, Andréanne Leblanc, Julien Lamontagne, Meriem Messaoudene, Arielle Elkrief, Bertrand Routy. *CRCHUM, Montreal, Canada*

Background The gut microbiome plays a key role in immune checkpoint inhibitors (ICI) efficacy and several strategies are currently being investigated to improve microbiome composition. The impact of a specific diet on microbiome modulation and clinical outcomes remains unknown. In this study, we assessed the effects of a high fiber diet on clinical outcomes as well as on microbiome composition, production of fecal metabolites, and intratumor immune infiltration in metastatic non-small cell lung cancer (mNSCLC) patients amenable to ICI.

Methods In this prospective study, 39 chemotherapy-refractory or naive patients with mNSCLC treated with ICI alone or in combination with chemotherapy completed a validated dietary survey. Based on the total fiber intake, patients were divided into high vs low fiber groups (HF vs LF). Objective response rate (ORR), progression-free survival (PFS) and overall survival (OS) were compared between both groups. In addition, fecal and tumor samples were collected prior to ICI initiation. Fecal metagenomic sequencing was performed and fecal short-chain fatty acids (SCFA) were measured by LC-MS/MS. Tumoral transcriptome profiling was performed through RNA sequencing to define differentially expressed pathways.

Results Baseline characteristics were well balanced between both groups, including body mass index (BMI) and PD-L1 status. Median PFS for the HF group was longer compared to the LF group (27.4 vs 12.6 months). Microbiome metagenomic profiling revealed higher baseline alpha diversity ($p=0.048$) in the HF group compared to the LF group. Bifidobacterium, Alistipes, and Bacteroides salyersiae were enriched in the HF group while Fusobacterium was overrepresented in the LF group. SCFA measurement revealed that a high level of propionate correlated with a significantly longer OS (not reached vs 18.4. months, $p=0.02$) in the entire cohort. Moreover, propionate levels were significantly higher in the HF vs LF group ($p=0.02$). At the tumor level, RNA sequencing demonstrated a downregulation of DNA repair mechanisms and an upregulation of humoral and adaptive immune responses in the HF group.

Conclusions In this study, we demonstrated that a HF diet in patients with mNSCLC was associated with better clinical outcomes. Importantly, HF was associated with an enrichment of previously reported beneficial gut bacteria. Moreover, propionate correlated with longer OS and was increased in the HF group. This study provides further insights into how the diet can beneficially shift the microbiome composition and intratumor immune responses in patients with mNSCLC treated with ICI and this may lead to novel, dietary-geared therapeutic avenues in the oncomicrobiome arena.

Ethics Approval The study was approved by CRCHUM Institution,s Ethics Board, approval number 17.035.

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680 AUTOMATED ION TORRENT BASED SOLUTION ENABLES ACCURATE GUT MICROBIOME QUANTIFICATION OF BACTERIAL SPECIES RELEVANT TO RESEARCH IN CANCER AND ITS RESPONSE TO IMMUNOTHERAPY

Shrutii Sarda*, David Merrill, Heesun Shin, Anna McGeachy, Birgit Drews, Wing Lee, Rajesh Gottimukkala, Janice Au-Young, Fiona Hyland. *Thermo Fisher Scientific, South San Francisco, USA*

Background A low-cost targeted solution to profiling gut microbial diversity is sequencing of the 16S rRNA gene; however, it is often insufficient to gain species level resolution due to high homology across different bacteria. Therefore, we developed a first-of-its-kind targeted sequencing solution that supplements 16S gene targets, with highly species-specific primers for a cohort of 73 bacteria associated with research in diabetes, cancer and its response to immunotherapy,

gastrointestinal and auto-immune disorders. This assay performs at 100% sensitivity and specificity for the species-level detection (Ion AmpliSeq Microbiome Health Research Kit: www.thermofisher.com/ngsmicrobiome) of these bacteria and is hence better suited for gut microbiome profiling in the context of the above phenotypes, as compared to other existing solutions.

Methods To assess the utility of the panel in cancer immunotherapy research, we sequenced DNA from 15 stool samples from subjects with Non-Small Cell Lung Carcinoma (NSCLC) undergoing immunotherapy, and compared their microbiome profiles to 26 healthy stool samples collected internally. With our post-sequencing workflow on Ion Reporter™, we automatically generate a report with taxonomic classifications, sample diversity metrics through QIIME2 integration, and relative abundance visualizations for bacteria across multiple samples.

Results We identified significant microbiome composition differences between the healthy samples and cancer/treated samples, as evidenced by (i) a clear separation between the two cohorts based on a beta diversity principal coordinate analysis (PCoA) plot, driven by large abundance changes in Clostridium, Lachnoclostridium, Subdolinigranulum and Oscillibacter ($P < 0.05$), (ii) grouping into distinct classes based on overall microbiome profiles (Analysis-of-Similarities ANOSIM $P = 0.003$), and (iii) differences in abundances of specific bacteria with anti-tumor effects[1] such as F. prausnitzii ($P = 0.02$).

Conclusions We have created a highly multiplexed, sensitive and specific assay for robust characterization of gut microbiota, with compatibility on both (i) the Ion GeneStudio S5™ with a 48-hr sample-to-result turnaround, and (ii) the new Ion Genexus™ System, a fully integrated platform featuring a hands-off, automated sample-to-report workflow that delivers results in a single day. It enables an efficient and affordable means for conducting extensive analyses of the human microbiome having applications in the study of phenotypic variability, and the potential relationship to disease.For research use only. Not for use in diagnostic procedures.

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681 SINGLE PIPELINE RE-ANALYSIS REVISES MICROBIOME ASSOCIATIONS WITH ANTI-TUMOR RESPONSE TO CHECKPOINT INHIBITORS

[1]Fyza Shaikh*, [2]James White, [1]Joell Gills, [3]Taiki Hakozaki, [4]Richard Corentin, [4]Bertrand Routy, [5]Yusuke Okuma, [6]Mykhaylo Usyk, [6]Abhishek Pandey, [6]Jeffrey Weber, [6]Jiyoung Ahn, [1]Jarushka Naidoo, [1]Evan Lipson, [1]Drew Pardoll, [1]Cynthia Sears. *[1]Johns Hopkins School of Medicine, Baltimore, MD, USA; [2]Resphera Biosciences, Baltimore, MD, USA; [3]Tokyo Metropolitan Cancer and ID Center, Tokyo, Japan; [4]University of Montreal Research Center, Montreal, Canada; [5]5Tokyo Metropolitan Cancer and ID Center, Tokyo, Japan; [6]NYU School of Medicine, New York, NY, USA*

Background Several studies suggest the gut microbiome may be a novel, modifiable biomarker for clinical efficacy of immune checkpoint inhibitors (ICIs). Microbiome profiling of pre-treatment samples demonstrated that high alpha-diversity and enrichment of specific bacterial species are associated with improved tumor responses in melanoma, renal cell cancer (RCC), and non-small cell lung cancer (NSCLC). Despite these reports, the specific bacteria or communities helpful or harmful have been inconsistent across study populations, and further correlation with immune and mutational biomarkers are limited or lacking. We hypothesize that, by use of a larger sample size and a consistent computational approach, we would derive a clearer microbial profile that correlated with immunotherapeutic outcomes.

Methods We re-analyzed the available raw 16S rRNA amplicon and metagenomic sequencing data across five recently published ICI studies (n=303 unique patients) of responder (R) and nonresponse (NR) using a consistent computational approaches (Resphera Insight and MetaPhlAn2). Using novel microbiota signatures, we identified Re-analysis Indices for R- and NR-associated bacteria and validated the result in three addition cohorts with available raw sequencing data in patients with melanoma, hepatocellular cancer (HCC), and NSCLC (n=105).

Results Our results confirm signals reported in each study, though some bacteria reported initially were not statistically significant after correction for false discovery rate. Likely, in part, because our analysis allows for comparison of individual species across cohorts, we were able to identify new bacterial signatures, such as Oxalobacter formigenes, Roseburia hominis and Veillonella parvula, Clostridium hathewayi, enriched in R and NR respectively. When our Re-analysis Index was compared to an index assembled from the literature, we noted improvement occurred in a sensitivity and specificity analysis, especially in NR-associated signals. Moreover, we found that alpha-diversity was not consistently predictive of response or nonresponse to ICIs. Our Re-analysis Index also validated in melanoma patients and HCC but did not perform as well in the NSCLC cohort, suggesting the need for further refinement based on tumor type.

Conclusions In summary, this bioinformatics platform improves on existing pipelines by standardizing critical preprocessing and downstream analysis tools, enabling comprehensive evaluations of taxonomic and functional signals across sequencing datasets. Notably, the NR-associated Re-analysis Index shows the strongest and most consistent signal using a random effects model and in a sensitivity and specificity analysis ($p < 0.01$). Our integrated analyses suggest an approach to identify patients who would benefit from microbiome-based interventions targeted to improve response rates by using a biomarker for nonresponse.

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Novel single-agent immunotherapies

682 ANTIBODY-MEDIATED BLOCKADE OF INTERLEUKIN-10 RECEPTOR-ALPHA PROMOTES THE ACTIVATION OF IMMUNE CELLS FROM IN VITRO DISSOCIATED TUMOR SAMPLES

Alexey Berezhnoy*, Kalpana Shah, Daorong Liu, Peter Lung, Vatana Long, Kurt Stahl, Douglas Smith, Francine Chen, Hua Li, Jonathan Li, Ralph Alderson, Valentina Ciccarone, James Tamura, Ezio Bonvini, Gundo Diedrich, Paul Moore. *MacrGenics, Inc., Rockville, MD, USA*

Background Interleukin-10 (IL-10) is a multifunctional cytokine that can mediate immune suppression or activation depending on the immunological context. Mouse studies have demonstrated that blockade of IL-10 enhances immune response against tumors and chronic viral infections;[1] [2] intriguingly,