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641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

Gut Microbiome Links to Disease Stage and Immune Microenvironment in Patients with Chronic Lymphocytic Leukemia

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Background
Recent studies have shown extensive crosstalk between our immune system and the gut microbiome (GM). The host immune system plays a vital role in the maintenance of GM homeostasis by establishing a balance between eliminating invading pathogens and promoting the growth of beneficial microbes, whereas GM can modulate the immune system by cytokine production. As chronic lymphocytic leukemia (CLL) is characterized by a high rate of infectious complications and an altered immune microenvironment, it is warranted to uncover the link between microbiome composition and immune dysregulation in CLL patients. Thus, we investigated the microbiome pattern and potential connections to disease characteristics and phenotypes and distribution within the immune microenvironment in CLL patients requiring treatment.

Methods
Blood and feces samples were collected from 17 patients with CLL prior to targeted treatment with acalabrutinib or ibrutinib+venetoclax in clinical trials. T-cell maturation subsets, Treg/Th17 subsets, and activation- and exhaustion profiles were assessed in fresh blood samples by an 8-tube, 10 color flow cytometry panel (DuraClone), including one tube containing beads for calculating absolute concentrations. Fecal samples were subjected to shotgun sequencing using Illumina HiSeq and taxonomical profiling was done using an in-house bioinformatics pipeline. The bioinformatics analysis involved microbial species abundances comparison and co-occurrence identification across patient clusters. Electronic health records provided data on clinical baseline characteristics. Case report forms and data on use of antimicrobial drugs will be assessed at a later stage to investigate potential associations with infections or toxicity related adverse events. All patients provided written informed consent and the study was approved by the National Ethics Committee and the Data Protections Agency.

Results
Unsupervised clustering analyses based on GM composition data revealed two GM clusters, where we found an overlap constituting approximately 40% of the cohort between Binet stage C (7/10 patients) and GM cluster 2 (7/10 patients) (Figure 1A). GM cluster 2 was characterized by differentially abundant species such as *Bacteroides fragilis* and *Eggerthela lenta*, demonstrating the two highest fold change and elevated relative abundances (Figure 1B). Closer examination of the taxonomic composition characteristic for the 7 patients revealed a small set of indicator microbiome species, where *Clostridium innocuum* together with *Clostridiales sp., Bacteroides fragilis and Escherichia coli*, were the most significantly abundant co-occurring species for CLL patients with Binet C. Preliminary examination of T-cell subset data showed generally lower concentrations of T-cells in patients from GM cluster 2 compared to GM cluster 1, where the 7 patients overlapping with Binet C furthermore

had decreased levels of Th17 CD4+ and Tc17 CD8+ cells compared to both the normal reference level and the rest of the cohort (Figure 1C).

Conclusion

Our preliminary results suggest a link between CLL stage at time of needing treatment, gut microbiome composition, represented by a subset of highly abundant bacterial species, and T-cell distribution with reduced Th17 and Tc17 subsets, to be further investigated. To our knowledge, we are thus among the first to present data connecting the gut microbiome to tumor microenvironmental changes in patients with CLL. Moving forward we will further explore microbiome-microenvironment interactions with focus on the production of short-chain fatty acids (bacterial metabolites which play crucial roles as immunomodulatory molecules) to investigate connections between microbiome, immune phenotypes, and clinical outcomes including infections, treatment-related toxicity, and disease progression, to light on novel avenues for therapeutic interventions in CLL.

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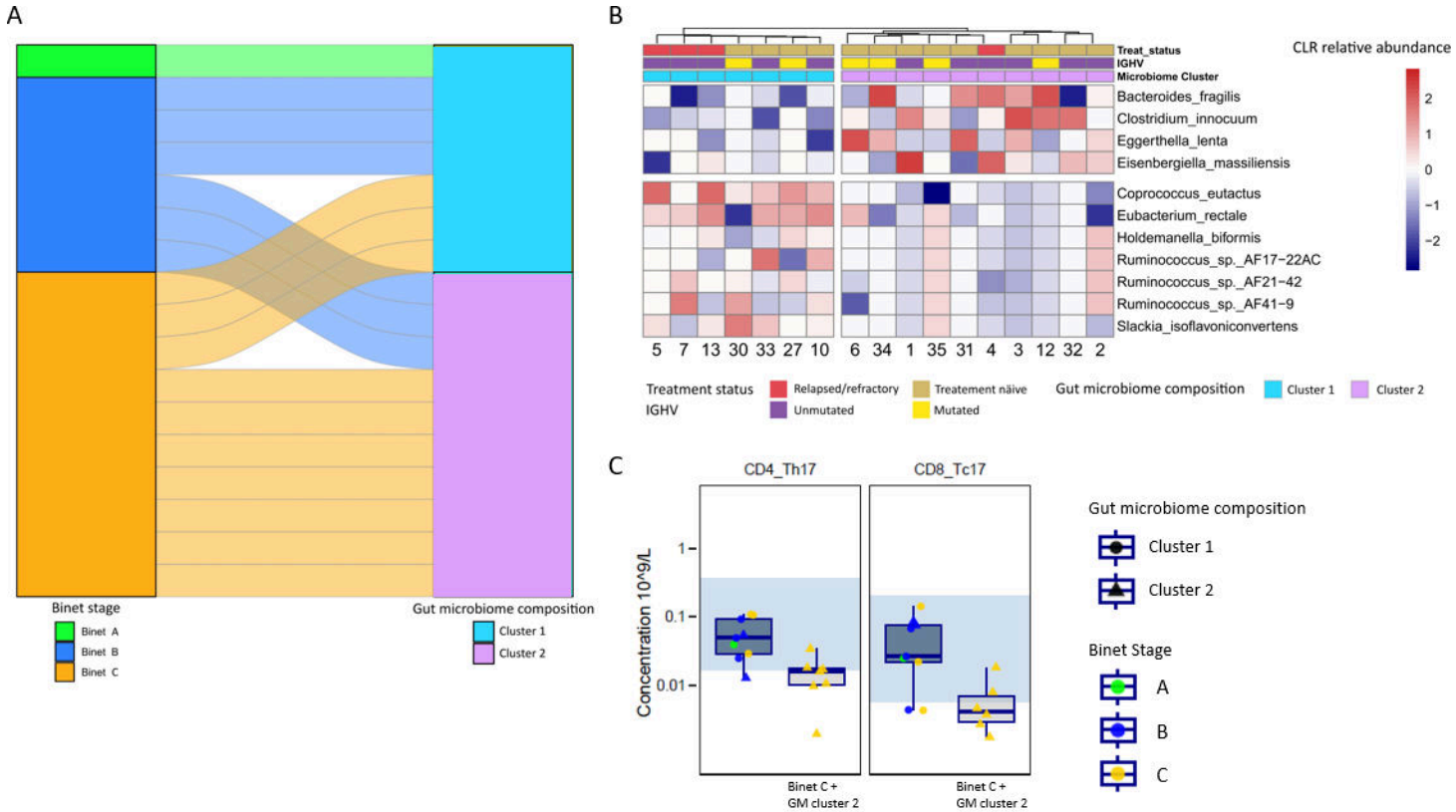

Figure 1. A) Alluvial plot where each line represents one patient. Color legend indicates Binet stage and gut microbiome compositions based on unsupervised clustering of β-diversity (Cluster 1, Cluster 2). **B)** Heatmap of differentially abundant bacterial species (fold change > 1.5 and p-value < 0.01) between Clusters 1 and 2. The clusters were formed by hierarchical clustering using ward.D method applied on full dataset consisting of 445 species. Color scale: Centered log ratio transformed relative abundance of bacterial species, scaled by columns in *pheatmap* function in R. **C)** Boxplots presenting concentration levels (on a $\log_{10}$ axis) of CD4+ Th17 T-cells and CD8+ Tc17 T-cells in the 7 patients with Binet C overlapping with GM cluster 2 vs the rest of the cohort. Shaded areas represent the normal reference level.

Figure 1