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P1430 GUT MICROBIOTA DIVERSITY AND BUTYRATE PRODUCERS IMPACTS ON NON-HODGKIN LYMPHOMA PATIENTS RESPONSE TO CD19 CAR-T THERAPY

Topic: 24. Gene therapy, cellular immunotherapy and vaccination - Biology & Translational Research

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Background: The gut microbiota is involved in the regulation of normal hematopoiesis and the correct development of the immune system. Particularly butyrate, a short chain fatty acid (SCFA) derived from the microbial fermentation of dietary fibers, affects immune cell proliferation, activation, apoptosis and cytokine expression. Recent studies have focused on understanding the microbiota as a modulator of the initiation and progression of hematological neoplasms. In addition, gut microbiota plays a key role in the efficacy and toxicity of antitumor treatments, especially immunotherapy.

Aims: The aim of this study is to analyse the prognostic role and mechanism of action of the gut microbiota, and specifically of SCFAs, in NHL patients treated with CD19 CAR-T.

Methods: Stool and serum samples were collected from NHL patients at the time of screening prior to CAR-T treatment, categorizing them according to the primary response. Microbial DNA was extracted with the AllPrep PowerFecal DNA/RNA kit and 7 hypervariable regions of the 16S rRNA gene were sequenced using the Ion 16S Metagenomics kit on an Ion S5 System. The sequences were analysed using QIIME2 and the SILVA138 database. PICRUSt2 was used to predict metagenomic functions based on as Kyoto Encyclopaedia of Genes and Genomes (KEGG) ortholog. SCFAs concentration were measured by LC-MS in an LC-ESI-QQQ 8030 (Shimadzu) instrument after acetonitrile precipitation and derivatization. The antitumor potential of SCFAs was evaluated in NHL human (DoHH2) and murine (A20) cell lines through the elaboration of dose-response curves after exposure to 48 hours. Finally, molecular pathways altered were analysed by western blot or qPCR in DoHH2 treated with butyrate 1mM.

Results:

Responder patients exhibited higher microbial richness, equity and α-diversity indexes than non-responder´s ones (Fig 1A). The β-diversity analysis determined the existence of a significantly different microbiome between both groups (Fig 1B).

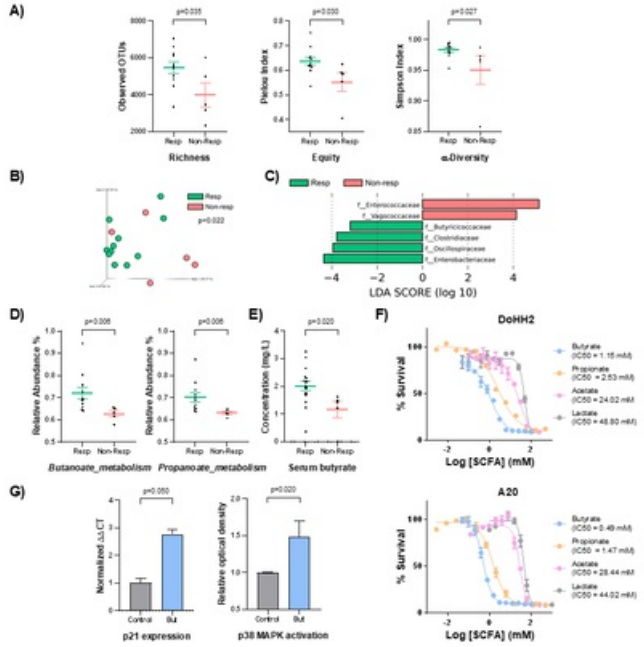
Interestingly, the taxonomic comparison revealed a significantly higher relative abundance of butyrate producer groups as *Butyricicoccaceae* (p=0.008), *Clostridiaceae* (p=0.009) and *Oscillopiraceae* (p=0.042) families in responders (Fig 1C). Indeed, the metagenomic analysis, which allows inference of the functional profile, revealed that the microbiome of responders is enriched in metabolic pathways related to the fatty acid metabolism, especially butyrate and propionate (Fig 1D). Similarly, serum butyrate levels were higher in responders. (Fig 1E). Recently it has been shown that *in vitro* pre-treatment of cytotoxic lymphocytes with this metabolite increases the antitumor cellular immunity in the context of adoptive immunotherapy (Luu et al., *Nat Commun* 2020).

Finally, supplementing this property of butyrate we have also characterized a direct effect on tumour cells. Butyrate and other SCFAs inhibit *in vitro* lymphoma cell growth in the millimolar range (Fig 1F) by the dysregulation of

MAPK pathway, but also by the enhanced expression of p21 (Fig 1G).

Image:

Figure 1. (A) Graphs of mean ± SEM of richness, equity and α-diversity indexes of the microbiota of Responder (Resp) and Non-responder (Non-resp) patient (n=16). **(B)** β-diversity of Resp and Non-resp patients (n=16) represented by principal coordinate analysis (PCoA) based on the Bray-Curtis distance. **(C)** LEfSe (*Linear discriminant analysis Effect Size*) analysis of abundance of microbiota families. **(D)** Relative abundance of KEGG metabolic pathways predicted in the patients microbiome. **(E)** Butyrate content in serum samples from patients (n=24). **(F)** Dose-response curves of non-Hodgkin lymphoma human (DoHH2) and murine (A20) cell line for microbiota SCFAs. **(G)** p38 MAPK activation (phosphorylated-p38/p38) determinate by western blot and p21 expression by qPCR.



Summary/Conclusion: These preliminary results reveal a higher diversity and a different microbiome profile in the gut microbiota of NHL patients that response to CD19 CAR-T cell therapy. Particularly, the taxonomic analysis, and subsequent validation in patient samples, reveals that butyrate is an important metabolite that may impact in the treatment response. Finally, the antitumor potential of this bioactive SCFAs found could promote their use as adjuvant therapy or chemical modifier of CAR-T.