Does microbiota composition act as predictive signature for the evaluation of chemoimmunotherapy response efficacy?

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Esophageal squamous cell carcinoma (ESCC) accounts for about 90% of all esophageal cancer cases and is the sixth most common cause of cancer-related death worldwide (1). The remaining quota consists of esophageal adenocarcinoma (EAD), and it differs in epidemiology, etiology, pathology, tumor location, and prognosis. The ESCC, usually localizes at the tracheal bifurcation, and has a poorer prognosis than EAD (2). Regarding epidemiology, ESCC is most common in Eastern Europe and Asia, while EAD is in North America and Western Europe (3). Tobacco and alcohol consumption are major risk factors for ESCC, while tobacco alone is a moderate risk factor for EAD (4). Furthermore, there are several histological subtypes among ESCC and EAD, which differ in metabolism, clinical features, cytokines, therapy outcomes, immune response, and tumor microenvironment (TME) (5).

ESCC treatment includes a multidisciplinary approach ranging from surgery, chemoradiotherapy as well as immunotherapy (6). Regarding the immunotherapy treatments, it has been documented that neoadjuvant chemoimmunotherapy (NACI) significantly favors the achievement of a complete response for resectable ESCC. Several studies have shown that NACI could become a promising treatment for locally advanced ESCC (7,8), but unfortunately not all ESCC patients adequately respond to NACI (9,10). This raises the question about identifying predictive biomarkers and potential mechanisms for assessing response to NACI in ESCC patients.

Nowadays it is established that variability in microbial communities among humans has a relevant impact on cancer phenotypes and response to therapy (11,12). Several studies have highlighted the key role of the gut microbiota (GM) in influencing the anti-tumor responses to chemotherapeutic agents and immunotherapies focusing on its ability to activate the intestinal immunity (13-15). Moreover, various bacteria has been found in ESCC and EAD patients, strengthening the link between intratumoral microbiota (ITM) and tumor signatures such as stage, and survival status (16).

However, the role of the microbiota in ESCC tumors remains to be further investigated.

In this scenario, recently Wu *et al.* explored the role of ITM composition in mediating the treatment response to NACI in patients with ESCC (17). Among them, 25 patients were treated with NACI and 15 were not treated. The ITM analysis revealed that there were no significant differences in the α- and β-diversity between the tumor and the adjacent normal tissue and between the NACI

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and untreated group. However, they found a significant difference in the β-diversity of the bacterial communities among responders and non-responders to NACI. These results were in line with previous findings in non-small cell lung cancer (NSCLC) and renal cell carcinoma (RCC), in which Routy *et al.* observed that cancer patients undergoing antibiotic treatments, did not respond to anti-programmed cell death 1 (PD-1) immunotherapy, proving a causal effect of microbes in modulating the response to therapy (18). In addition, the authors demonstrated that fecal microbiota transplantation (FMT) from feces of responder patients into mice, restored NACI antitumor activity (18). The efficacy of NACI treatment is positively correlated to a high concentration of intratumoral CD103$^+$ CD8$^+$ in head and neck squamous cell carcinoma (HNSCC) patients, as observed by Ren *et al.* (19).

In Wu *et al.* study, NACI responders showed a significantly different ITM signature compared to NACI non-responders in tumor tissue samples. At phylum level, a predominance of Firmicutes and Bacteroidota, and at genus level of *Actinomyces*, *Abiotrophia*, *Granulicatella*, and *Streptococcus* was observed (17). In fact, there was a positive correlation between *Streptococcus* abundance and immune tissue infiltration of CD8$^+$ T cells and granzyme B$^+$ (GrzB$^+$) and an anti-correlation with CD4$^+$ T cells and forkhead box P3 (FOXP3$^+$) cells. The positive association between *Streptococcus* and CD8$^+$ T polarization and secreted GrzB+ has been previously observed in oral squamous cell carcinoma (OSCC) patients compared to controls (20). By expressing an abundance of cytotoxicity cells within the TME, the efficiency of NACI treatment could be increased.

Single cell-RNA sequencing (scRNA-seq) revealed that non-responders tumor tissues had a decreased proportion of cytotoxic T cells with an increase of FOXP3$^+$ and cytotoxic T-lymphocyte antigen 4 (CTLA4) (17). FOXP3$^+$ and CTLA4 are notably markers of an immunosuppressive microenvironment.

All these results were further confirmed in a mouse model of tumorigenesis obtained by subcutaneous implantation of mouse esophageal cancer cells (mEC25). In those mice, previously treated with antibiotics, a FMT was performed using stool samples obtained from ESCC patients (non-responders or responders) or from healthy controls (HCs) donor. Mice treated with FMT from responders, showed a decreased tumor growth and the 16S rRNA sequencing revealed that *Streptococcus* was present and positively correlated with GrzB$^+$ and CD8$^+$ T cells in ITM. On the contrary with HC donors and non-responders FMT,

the mouse showed no positive correlation with the presence of GrzB$^+$ and CD8$^+$ T cells, suggesting a causal relationship between *Streptococcus* and immune cells infiltration.

In addition, a different tumorigenesis murine model previously treated with antibiotics, repopulated with *Streptococcus* clones isolated from tumor tissue of NACI responder's patients was set up. Mice were treated with anti-PD-1 immunotherapy causing an intratumoral increase of immune cell infiltration and an enhanced immunotherapy response. To establish a causal relationship between *Streptococcus*, immune cells infiltration and immunotherapy response, *Streptococcus* was depleted after antibiotics administration, causing a decrease of the response to anti-PD-1 as well as the intratumoral infiltration cells. The same results were not achieved with the use of *Escherichia coli* instead of *Streptococcus* (17). Accordingly, Peng *et al.* analyzed the microbiota in stool samples of 74 patients with stage III and IV gastrointestinal (GI) cancer receiving anti-PD-1/ programmed death-ligand 1 (PD-L1) treatment and they found a positive association of *Eubacterium*, *Lactobacillus*, and *Streptococcus* and anti-PD-1/PD-L1 response (21). Also, Baruch *et al.* found that patients affected by refractory melanoma receiving FMT from donor responders to anti-PD-1 therapy, became responders to a second cycle of anti-PD-1 therapy (22). Moreover, fecal analysis showed a higher relative abundance of *Enterococcaceae*, *Enterococcus*, and *Streptococcus australis* in responder patients (22). However, different results were found in a meta-analysis about melanoma patients treated with anti-PD-1, where the authors found that the GM dominated by *Streptococcus* spp. was not enhancing anti-cancer immunity and might induce organ dysfunction (23). These discrepancies on different results could be likely due to different tumor types and subtypes, ethnicities, lifestyle, and number of patients enrolled.

Collectively, these interesting data suggest that gut-derived *Streptococcus* may migrate in the bloodstream, and then colonize the intratumoral tissue, and its presence can (I) influence the microorganism's composition; (II) modulate the immune infiltration within the TME; and (III) improve the immunotherapy outcome.

Therefore, Wu *et al.* manuscript presented a cutting-edge work that paves the way in the future for the identification of microbiotic signatures for each tumor type and stage (17). These signatures could be used as predictive prognostic features of several other cancers and as biomarkers of immunotherapy responsiveness for patients. Moreover, FMT enriched with *Streptococcus* could be a promising

treatment to restore the immune response in patients who were previously non-responders to immunotherapy. In terms of FMT safety, healthy donors have to be negative in a series of serological and microbiological screening tests, alongside there are also ongoing attempts to create more standardized procedures using synthetic bacterial preparations such as "Bacterial Consortium" (24).

However, to reach these goals, more clinical studies are needed to better define the role of microbiota signatures as biomarkers and to tailor interventions to ESCC specific histologic subtypes.

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Footnote

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