Old Herborn University Seminar Monograph 36: The microbiome and cancer.
A summary of the lectures given during the 36th Old Herborn University Seminar
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MICROBIOTA-CENTRED INTERVENTIONS IN IMMUNO-ONCOLOGY

Introduction

The topic of the presentation given by *Dr. Meriem Messaoudene* (Hospital Research Centre (CRCHUM), University of Montreal, 1560 Rue Sherbrooke E, Montreal, Canada) was "Microbiota–centred interventions in immuno-oncology".

She started with the statement that the gut microbiome has emerged as one of the hallmarks of cancer in addition to the already recognised hallmarks (Figure 1).

The polymorphic gut microbiome can harbour both beneficial and harmful bacteria. Its composition and function can be modulated by genetics, lifestyle and therapeutic measures including diet, probiotics, prebiotics, and in some cases faecal microbiota transplantation (FMT).

Negative influences on the microbiome

However, some interventions can also have a negative impact on the gut microbiome. In particular, the use of antibiotics can eliminate beneficial bacteria, thereby allowing harmful bacteria the possibility to expand. It was shown in patients with advanced non-small-cell lung cancer (NSCLC) treated with immune checkpoint inhibitors (ICI) for their cancer, that antibiotic exposure has been associated with altered gut microbiota composition and reduced effectiveness of ICI treatment (Figure 2; *Derosa* et al., 2018).

Routy and colleagues showed also that antibiotics inhibited the clinical benefit of ICIs in patients with advanced cancer. In functional transfer experi-

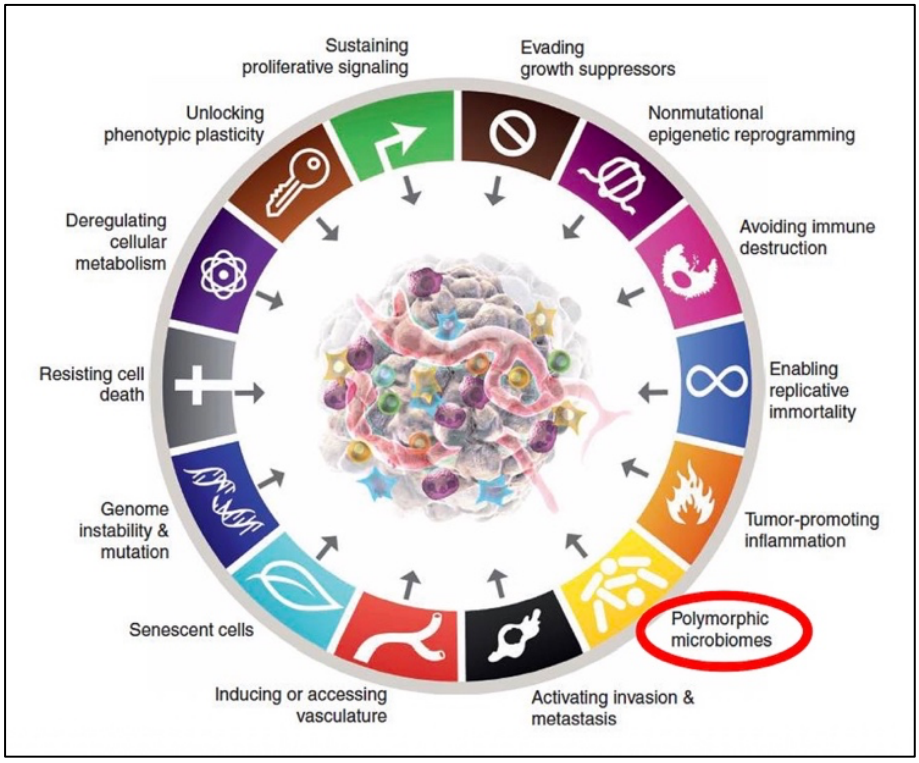

Figure 1: The hallmarks of cancer include the polymorphic microbiome. (Figure adapted from *Hanahan*, 2022).

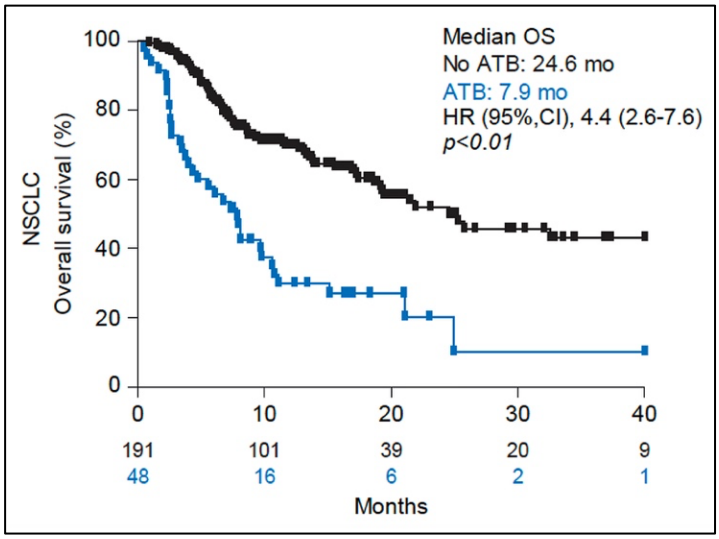

Figure 2: Overall survival in patients with NSCLC treated with ICI, stratified by use of antibiotics within 30 days of initiating ICI. (Figure from *Derosa* et al., 2018).

ments, faecal microbiota transplantation (FMT) from cancer patients who responded to ICIs into germ-free or antibiotic-treated mice ameliorated the anti-tumour effects of PD-1 blockade, whereas FMT from non-responding patients failed to do so (*Routy* et al., 2018). They further reported correlations between clinical responses to ICIs and the relative abundance of *Akkermansia muciniphila*, and showed that oral supplementation with *A. muciniphila* after FMT with nonresponder faeces restored the efficacy of PD-1 blockade.

When antibiotics are clinically unavoidable, a pragmatic approach is to use the narrowest effective spectrum for the shortest necessary duration, to minimize disruption of the microbiome and potential negative impact on immunotherapy.

The microbiome-protective effect of the antibiotic absorbent DAV132

Dr. Messaoudene reported on a study that was ongoing at the time of the seminar, and has since been published in Nature Communications (*Messaoudene* et al., 2024). In this study the authors tested a colon targeted absorbent (DAV132) given orally, which sequester antibiotics in the distal intestine and thereby limit microbiota damage. DAV132 is charcoal-coated formulated for ileo-caecum delivery, enabling adsorption of residual antibiotics before they reach the colon in active form, reducing the risk of dysbiosis.

The DAV132 colon-targeted absorbent was tested in a randomised phase I clinical trial with 72 healthy volunteers. Two doses of DAV132 were tested. The study consisted of three different arms. The first arm included a group of healthy volunteers who did not receive antibiotics with or without DAV132. The volunteers in the second arm were administered ceftazidime-avibactam (CZA), which is one of the most common antibiotics administered to patients, with or without DAV132. And finally, the third arm consisted of healthy volunteers treated with piperacillin-tazobactam (PTZ) alone, or in combination with DAV132 (Figure 3).

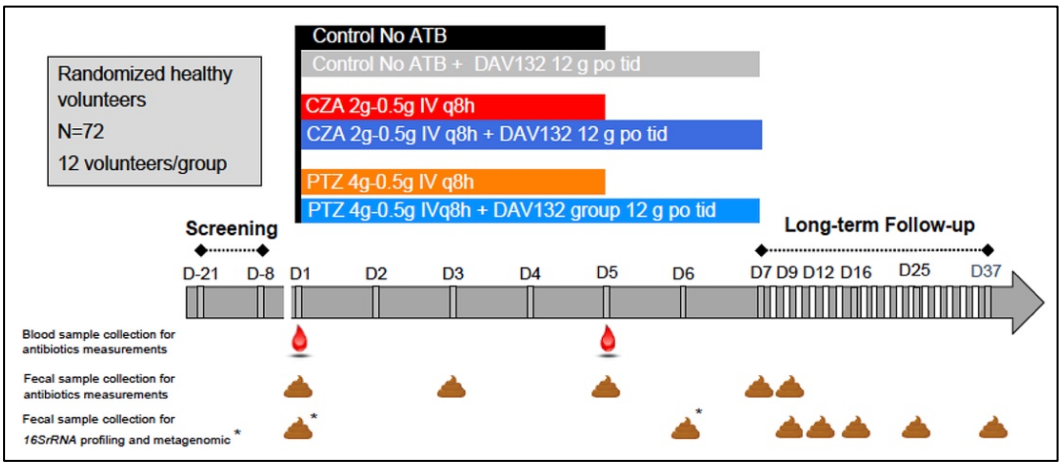

Figure 3: Clinical trial design and pharmacokinetics of antibiotics in the plasma and faeces of healthy volunteers (HV). (Figure from *Messaoudene* et al., 2024).

Antibiotics were administered intravenously for 5 days, while DAV132 was administered orally 12 g three times a day for 7 days. Blood samples were collected at 2 timepoints to measure antibiotic concentration in the blood and faecal samples were collected at different timepoints to measure the antibiotic concentration and to perform 16SrRNA sequencing and metagenomics microbiome profiling. They found that DAV132 led to significant decrease in CZA or PTZ faeces concentration. When co-administered with antibiotics, DAV132 preserved microbiome diversity, accelerated recovery to baseline composition and protected key commensals. The authors concluded that DAV132 represents a promising strategy to mitigate antibiotic-associated dysbiosis and warrants evaluation in patients, including those receiving cancer immunotherapy.

The study also explored functional consequences for anti-PD-1 efficacy using donor-to-mouse transfer experiments. For this, they transplanted the stools from healthy volunteers from the clinical study treated with antibiotics alone or with antibiotics plus DAV132 into germ-free C57BL/6 mice. Two weeks after FMT, mice were implanted subcutaneously with 0.8×10^6 MCA-205 (mouse fibrosarcoma cell line) cells. When the tumours reached 25 to 35 mm^2 in size, mice were treated four times intraperitoneally every three days with anti-PD-1 monoclonal antibody (250 µg/mouse). Tumours were harvested 11 days after the first injection of anti-PD-1. The transplanted faeces from healthy volunteers treated with CZA or PTZ alone inhibited the anti-PD-1 response, whereas FMT from volunteers treated with antibiotics plus DAV132 preserved sensitivity to anti-PD-1. This supports the concept that microbiome protection during antibiotic exposure can maintain ICI responsiveness in recipient mice.

Flow cytometry analysis on the tumour of the different groups of mice suggested that the mice receiving FMT from the group CZA plus DAV132 after six days of treatment maintain a high ratio $CD8^+$ T cells compared to the mice receiving stools from the group treated only with CZA during six days. It was also observed that the mice receiving faecal transplants from the volunteers treated with PTZ plus DAV132 showed an increase of $CD8^+$ T cell population after treatment with anti-PD-1.

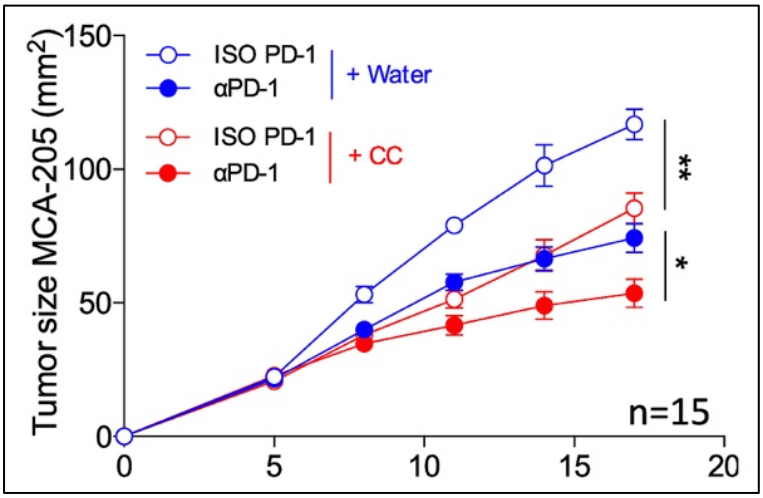

Figure 4: Tumour growth kinetics in SPF C57BL/6 mice after sequential injections of anti-PD-1 or iso-PD-1 and daily oral gavage with camu-camu or water in a MCA-205 sarcoma tumour model (n=15 mice/group). (Figure from: *Messaoudene* et al., 2022).

Overall, the study confirms that antibiotic-related dysbiosis is associated with a decrease of alpha diversity, a decrease of the "good bacteria" (like *Ruminococcus*) of which it is known that they are associated with a good response to immunotherapy. The antibiotic-related dysbiosis can result in an increase of the "bad bacteria" (like *Hungatella* and *Akkermansia*). When the microbiome is protected using DAV132, the alpha diversity is partially protected and can preserve the good bacteria such as *Ruminococcus*, *Eubacterium*, *Alistipes*, and *Faecalibacterium prausnitzii*. The protection of these bacteria seems to be associated with maintaining the immunotherapy response in mice.

These results are promising, but further studies are needed to evaluate the possible role of DAV132 in the treatment of cancer patients.

The influence of camu-camu on the gut microbiome

Dr. Messaoudene reported on a second study. Camu-camu, also known as *Myrciaria dubia*, is an Amazonian berry rich in phytochemicals and has been shown to exert protective prebiotic effects against obesity and related metabolic disorders in mice through increasing the abundance of *Akkermansia muciniphila* and *Bifidobacterium* in the gut (*Anhê* et al., 2019). In this second study, Messaoudene and colleagues evaluated whether the prebiotic action of camu-camu could also shift the gut microbiome in a way that improves antitumour immunity and responsiveness to immune checkpoint blockade. In the MCA-205 tumour model, known to be sensitive to anti-PD-1, they evaluated whether camu-camu could enhance anti-PD-1 efficacy. Figure 4 shows the tumour growth in mice treated with isotype control (iso-PD-1, the open blue dots) or with anti-PD-1 (filled blue dots), with (the open red dots) or without 1 (filled red dots) daily camu-camu. In addition, camu-camu alone showed antitumour activity comparable to anti-PD-1 in this setting. Importantly, combining camu-camu with anti-PD-1 further improved tumour control, with smaller tumour volumes over time.

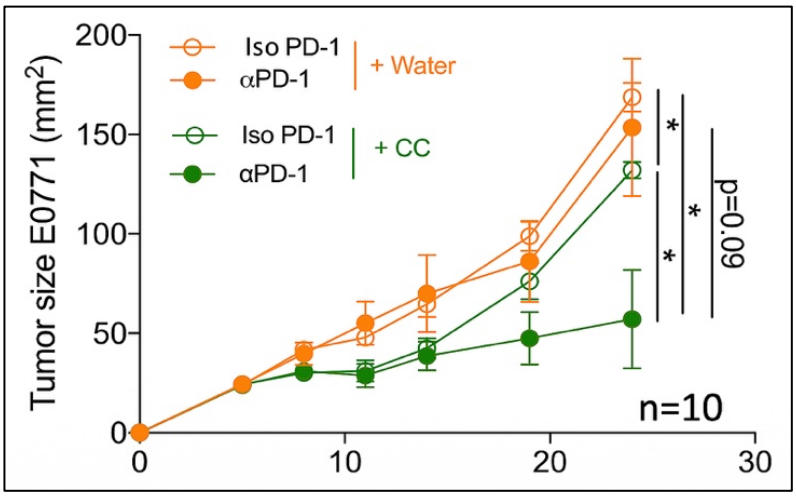

Figure 5: Tumour growth kinetics in SPF C57BL/6 mice after sequential injections of anti-PD-1or iso-PD-1 and daily oral gavage with camu-camu or water in a E0771 breast cancer model (n=10 mice/group). (Figure from: *Messaoudene* et al., 2022).

They tested camu-camu also in the anti-PD-1-resistant E0771 breast cancer model (n = 10 mice/group) and confirmed that the anti-PD-1 alone did not exhibit any anti-tumour activity in this model (Figure 5). Camu-camu alone had only a minimal effect, whereas camu-camu combined with anti-PD-1 transformed this resistant tumour model to a sensitive model. Camu-camu/iso-PD-1 demonstrated minimal anti-tumour activity, whereas the combination of camu-camu/anti-PD-1 increased the anti-PD-1 activity.

To identify the bio-active compound(s), camu-camu, was fractionated by High Performance Liquid Chromatography (HPLC), yielding 49 compounds. All the compounds were tested individually and only one compound, castalagin, showed the same effect as the complete camu-camu raw extract. Castalagin is a polyphenol also present in oak wood and is known to have anti-inflammatory properties.

The influence of castalagin on the gut microbiome

To test whether castalagin has the same effect as camu-camu, they performed FMT into germ-free mice, using faeces from non-small cell lung cancer patients known to be resistant to anti-PD-1. These patients were non-responders and performing FMT using faeces from these non-responder patients induces a resistance to anti-PD-1. After subcutaneous implantation of MCA-205 cells, they treated the mice with either the anti-PD-1 or the iso-PD-1 combined with castalagin administration by oral gavage or combined with water administration (controls). In the mice that were given anti-PD-1 and castalagin, a decrease in tumour size was observed when compared to the group that was treated with iso-PD-1 plus castalagin or the water control group (Figure 6).

The following step was performing a microbiome profiling of the mice faeces and they observed that the mice receiving castalagin had an increase in their faeces of *Ruminococcus*, *Akkermansia muciniphila*, *Ruminococcus*, *Blaucia*, *Alistipes* and other so called "good" bacteria, all being associated with a good response in patients treated with immunotherapy.

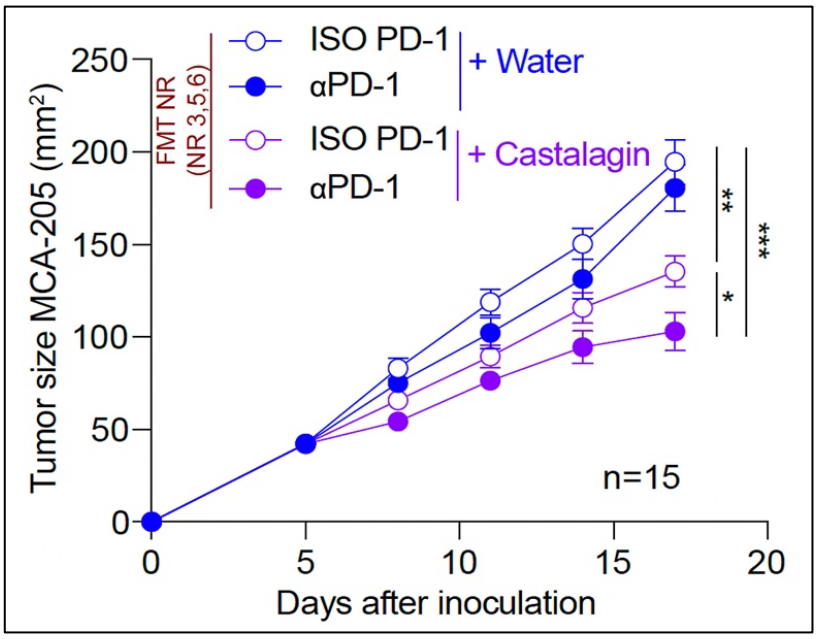

Figure 6: MCA-205 tumour growth kinetics in germ-free C57BL/6 mice after FMT from three non-responder patients with daily oral supplementation with castalagin or water in combination with anti-PD-1 or iso-PD-1.

They also performed an immune profiling using multiple technics including flow cytometry and immunofluorescence to test whether the response associated with the combination of castalagin with anti-PD-1 is also associated with a change of the immune system. They observed an increase of immune surrogate markers in the mice receiving castalagin including central memory $CD8^+$ T cells in the mice receiving castalagin. The percentage killing of CD8+ T OT-1 cells from the draining lymph nodes in mice treated with castalagin or water and immunized with CpG/OVA was also increased.

Dr. Messaoudene reported an ongoing clinical trial assessing camu-camu as a prebiotic adjunct to immune checkpoint inhibition in patients with non-small cell lung cancer and melanoma with very encouraging preliminary results.

In addition, preclinical results suggest that modulation of the gut microbiome by antibiotics is associated with ICI resistance. The faeces of two non-cancer patients enrolled in a clinical trial addressing the safety of oral administration of camu-camu were analysed. Preliminary faecal metagenomics revealed a positive trend in diversity and toward enrichment of *Ruminococcus bromii*, consistent with the results obtained in camu-camu-treated mice.

Faecal microbiome transplantation in the treatment of advanced cutaneous melanoma and advanced non-small cell lung cancer

The final part of the presentation focused on clinical FMT trials in advanced melanoma. Two independent phase I studies (*Davar* et al., 2021; *Baruch* et al., 2021) administered stool from complete responder melanoma patients treated with anti-PD-1 to patients resistant to anti-PD-1, resulting in clinical responses in approximately 25% of treated patients.

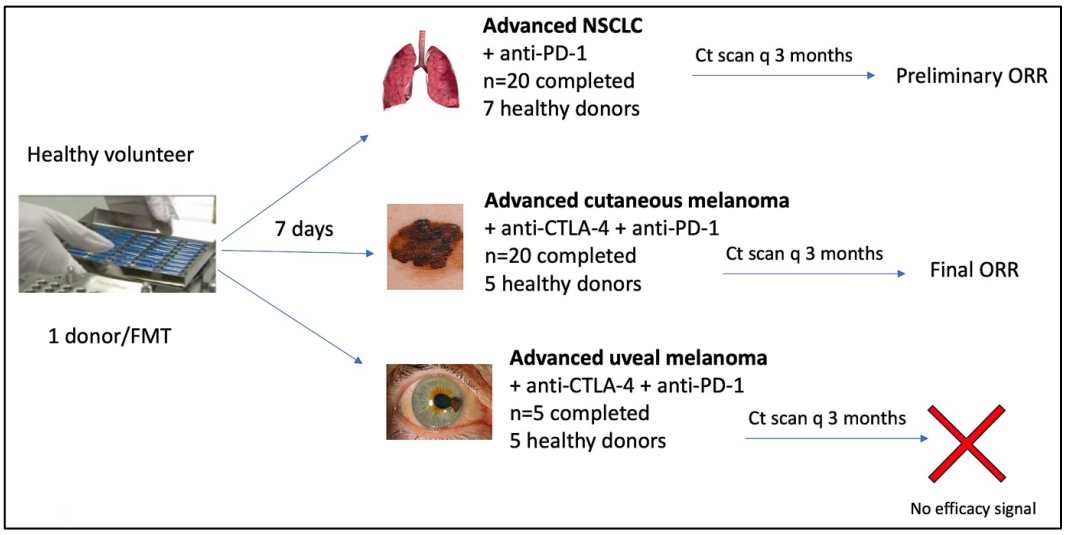

Figure 7: Clinical design of the FMT phase 1 study.

Based on these results, Dr. Messaoudene described a third phase I clinical trial (MIMICs) that they conducted in their lab at the Centre hospitalier de l'Université de Montréal (CHUM) (*Routy* et al., 2023). They did not use stools of complete responder patients, but stools of healthy volunteers. They enrolled 20 patients with cutaneous melanoma in this trial. They observed a 65% response compared to the 45% response that was obtained in the classical randomized phase 3 clinical trials. This was an increase in response of 20% in the patients treated with FMT plus anti-PD-1. Longitudinal microbiome profiling revealed that all patients engrafted strains from their respective donors. However, the acquired similarity between donor and patient microbiomes was only maintained over time in responders. Responders experienced an enrichment of immunogenic bacteria and a loss of deleterious bacteria following FMT. The responders maintained the microbiome of their donors, while in the non-responders the microbiome returned after one week to its original composition. The immune system of these patients was also analysed and they observed an increase of $ICOS^+$ $CD8^+$ T cells in the responders compared to the non-responders, consistent with enhanced anti-tumour immune activation.

Following the results of this MIMICs clinical trial, they started a second FMT clinical trial (FMT Luminate). Three different arms were included in this study (Figure 7). The first arm included advanced non-small cell lung cancer patients treated with anti-PD-1. The second arm included advanced cutaneous melanoma patients that were treated with anti-CTLA-4 plus anti-PD-1. This arm was also already completed with the enrolment of 20 patients. The third arm included advanced uveal melanoma patients treated with anti-CTLA-4 and anti-PD-1. However, after including five patients, this arm was closed because no signs of efficacy were observed in these patients.

Preliminary results in the non-small cell lung cancer patients, using FMT in combination with immunotherapy, showed an response rate of 72.2 %, an increase of about 30% compared to the

30% immunotherapy response in these patients.

The results in the advanced cutaneous melanoma cohort were also very good. They observed an increase in the objective rate response in these patients with about 20% compared to the classical randomized clinical trial using exactly the same clinical criteria.

In this study they observed one month after FMT in the responder group a decrease of the "bad bacteria", such as *Enterocluster*, and an increase of the "good bacteria", such as *Ruminococcus*, *Eubacterium*, and *Prevotella copri*.

The preliminary results of the last study at the time of the presentation of Dr. Messaoudene were:

- FMT alone has no safety signal and is acceptable for patients,
- Uveal melanoma is resistant to FMT + anti-PD-1 + anti-CLTA-4,
- Very strong preliminary results of FMT + anti-PD-1 +/- CTLA-4 in non-small cell lung cancer and metastatic cutaneous melanoma.

This paper was reviewed by Dr. Meriem Messaoudene before publishing.

LITERATURE

Anhê, F.F., Nachbar, R.T., Varin, T.V., Trottier, J., Dudonné, S., Le Barz, M., Feutry, P., Pilon, G., Barbier, O., Desjardins, Y., Roy, D., and Marette, A.: Treatment with camu camu (*Myrciaria dubia*) prevents obesity by altering the gut microbiota and increasing energy expenditure in diet-induced obese mice. Gut 68: 453-464 (2019).

Baruch, E.N., Youngster, I., Ben-Betzalel, G., Ortenberg, R., Lahat, A., Katz, L., Adler, K., Dick-Necula, D., Raskin, S., Bloch, N., Rotin, D., Anafi, L., Avivi, C., Melnichenko, J., Steinberg-Silman, Y., Mamtani, R., Harati, H., Asher, N., Shapira-Frommer, R., Brosh-Nissimov, T., Eshet, Y., Ben-Simon, S., Ziv, O., Khan, M.A.W., Amit, M., Ajami, N.J., Barshack, I., Schachter, J., Wargo, J.A., Koren, O., Markel, G., and Boursi, B.: Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science 371: 602-609 (2021).

Davar, D., Dzutsev, A.K., McCulloch, J.A., Rodrigues, R.R., Chauvin, J.M., Morrison, R.M., Deblasio, R.N., Menna, C., Ding, Q., Pagliano, O., Zidi, B., Zhang, S., Badger, J.H., Vetizou, M., Cole, A.M., Fernandes, M.R., Prescott, S., Costa, R.G.F., Balaji, A.K., Morgun, A., Vujkovic-Cvijin, I., Wang, H., Borhani, A.A., Schwartz, M.B., Dubner, H.M., Ernst, S.J., Rose, A., Najjar, Y.G., Belkaid, Y., Kirkwood, J.M., Trinchieri, G., and Zarour, H.M.: Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. Science 371: 595-602 (2021).

Derosa, L., Hellmann, M.D., Spaziano, M., Halpenny, D., Fidelle, M., Rizvi, H., Long, N., Plodkowski, A.J., Arbour, K.C., Chaft, J.E., Rouche, J.A., Zitvogel, L., Zalcman, G., Albiges, L., Escudier, B., and Routy, B.: Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. Ann. Oncol. 29: 1437-1444 (2018).

Hanahan, D.: Hallmarks of Cancer: New Dimensions. Cancer Discov. 12: 31-46 (2022).

Messaoudene, M., Pidgeon, R., Richard, C., Ponce, M., Diop, K., Benlaifaoui, M., Nolin-Lapalme, A., Cauchois, F., Malo, J., Belkaid, W., Isnard, S., Fradet, Y., Dridi, L., Velin, D., Oster, P., Raoult, D., Ghiringhelli, F., Boidot, R., Chevrier, S., Kysela, D.T., Brun, Y.V., Falcone, E.L., Pilon, G., Oñate, F.P., Gitton-Quent, O., Le Chatelier, E., Durand, S., Kroemer, G., Elkrief, A., Marette, A., Castagner, B., and Routy, B.: A Natural Polyphenol Exerts Antitumor Activity and Circumvents Anti-PD-1 Resistance through Effects on the Gut Microbiota. Cancer Discov. 12: 1070-1087 (2022).

Messaoudene, M., Ferreira, S., Saint-Lu, N., Ponce, M., Truntzer, C., Boidot, R., Le Bescop, C., Loppinet, T., Corbel, T., Féger, C., Bertrand, K., Elkrief, A., Isaksen, M., Vitry, F., Sablier-Gallis, F., Andremont, A., Bod, L., Ghiringhelli, F., de Gunzburg, J., and Routy, B.: The DAV132 colon-targeted adsorbent does not interfere with plasma concentrations of antibiotics but prevents antibiotic-related dysbiosis: a randomized phase I trial in healthy volunteers. Nat. Commun. 15: 8083 (2024).

Routy, B., Le Chatelier, E., Derosa, L., Duong, C.P.M., Alou, M.T., Daillère, R., Fluckiger, A., Messaoudene, M., Rauber, C., Roberti, M.P., Fidelle, M., Flament, C., Poirier-Colame, V., Opolon, P., Klein, C., Iribarren, K., Mondragón, L., Jacquelot, N., Qu, B., Ferrere, G., Clémenson, C., Mezquita, L., Masip, J.R., Naltet, C., Brosseau, S., Kaderbhai, C., Richard, C., Rizvi, H., Levenez, F., Galleron, N., Quinquis, B., Pons, N., Ryffel, B., Minard-Colin, V., Gonin, P., Soria, J.C., Deutsch, E., Loriot, Y., Ghiringhelli, F., Zalcman, G., Goldwasser, F., Escudier, B., Hellmann, M.D., Eggermont, A., Raoult, D., Albiges, L., Kroemer, G., and Zitvogel, L.: Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 359: 91-97 (2018).

Routy, B., Lenehan, J.G., Miller Jr., W.H., Jamal, R., Messaoudene, M., Daisley, B.A., Hes, C., Al, K.F., Martinez-Gili, L., Punčochář, M., Ernst, S., Logan, D., Belanger, K., Esfahani, K., Richard, C., Ninkov, M., Piccinno, G., Armanini, F., Pinto, F., Krishnamoorthy, M., Figueredo, R., Thebault, P., Takis, P., Magrill, J., Ramsay, L., Derosa, L., Marchesi, J.R., Parvathy, S.N., Elkrief, A., Watson, I.R., Lapointe, R., Segata, N., Haeryfar, S.M.M., Mullish, B.H., Silverman, M.S., Burton, J.P., and Maleki Vareki, S.: Fecal microbiota transplantation plus anti-PD-1 immunotherapy in advanced melanoma: a phase I trial. Nat. Med. 29: 2121-2132 (2023).