Extracellular vesicles delivered by PIPAC, as a biological nonovectorisation for antitumoral treatment and restoration of immunity in peritoneal digestive carcinomatosis.

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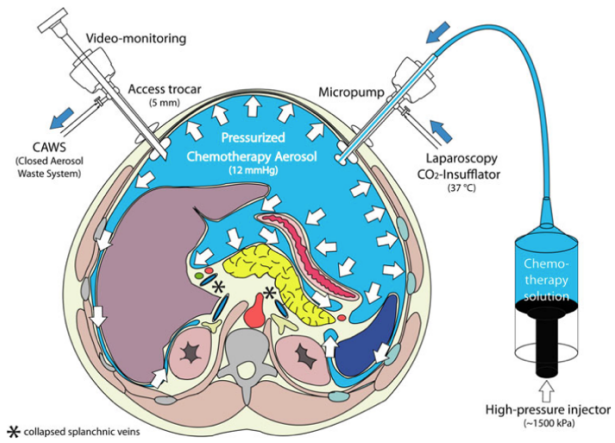
Issue, hypothesis and research work's main objective(s), expected results and impact:

Peritoneal surfaces are a common site for the dissemination of gastrointestinal malignancy. About two thirds of all peritoneal carcinomatosis (PC) are of digestive origin. Many patients afflicted by gastrointestinal malignancies will experience peritoneal tumor involvement during the disease progression : of these 40% from pancreatic cancer, 40% from gastric cancer and 20% from colorectal cancer. The prognosis of these patients is poor, and depends on the treatment possibilities. For example, median survival of patients with PC secondary to colorectal cancer, vary from 16 months with the best recent chemotherapy treatment [Franko J, et al Lancet Oncol. 2016;17:1709-1719], to 40 months with combined treatment involving complete cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) and systemic prolonged chemotherapy [Elias D, et al, J ClinOncol 2010;28: 63–8]. However, curative approach is reserved to a minority of selected patients, whereas most of patients are still treated with palliative intent. Different solutions are proposed for gastric cancers but survival results are limited. For pancreatic PC treatment is limited to palliative systemic chemotherapy. New therapeutic approaches should be applied to the majority of these patients.

Special attention is given to locoregional treatment because of the ability to achieve a high locoregional (peritoneal) drug concentration, while avoiding systemic toxicity [DakwarGR, et al Adv Drug Deliv Rev. 2017;108:13-24]. A recent technical innovation named PIPAC (Pressure Intra peritoneal Aerosol Chemotherapy) offers a new vision for PC treatment using mini invasive surgical approach, repeated intraperitoneal drug delivery and with the possibility to increase drug penetration using hyper pressure [Alyami M, et al Lancet Oncol. 2019;20:e368-e377].	

Today the protocol used in case of PC of digestive origin uses Oxaliplatin, Cisplatin or Doxorubicin. Conventional chemotherapeutics may however, rapidly leak from the peritoneal cavity and thereby result in negligible impact on cancer cells. We postulated that intraperitoneal drug delivery could change dramatically if cross-disciplinary research associating partner teams, from physical scientific fields and biomedical teams, can offer together new solutions. Projects of the Inserm unit U1275 are devoted to that cross-disciplinary research pathway.

The use of nanomedicines to prolong the residence time in the peritoneal cavity and to specifically target tumor cells, is being explored. Among the various types of nanovectorization, liposomes constitute the majority of the nanotherapeutics so far approved [Bregoli L, et al Nanomedicine. 2016;12:81-103]. In PC treatment, for two years, there has been a growing interest in liposomal nanovectorization of chemotherapy, microRNA,… [HiguchiT,et al Cancer Sci. 2019;110:2933-2940; Sugarbaker PH, et al Eur J SurgOncol. 2019; 29. pii: S0748-7983,Shariati M, et al Pharm Res. 2019 Jun 24;36(9):126, IizukaK , et al Drug Des DevelTher. 2018 Mar 29;12:673-683]. However, the results appeared contradictory in terms of tumoral drug penetration and stability. Bio-camouflaged and bio-inspired approaches are currently very attractive strategies in the emerging nanomedicine landscape for the delivery of drugs. In particular, extracellular vesicles (EVs) could represent a drug delivery vehicle of choice considering their endogenous properties of stability in blood circulation, immunotolerance and capacity to facilitate cell entry [Chulpanova DS, et al Front Pharmacol. 2018 Mar 20;9:259].

We have tested an innovative therapeutic management of PC that is bio-inspired and tumor-targeted by engineering mesenchymal stem cells (MSC) - EVs loaded with antitumoral drugs. In the specific context of tumor therapy, MSC present inherent tumor-trophic properties whose MSC-EVs could inherit. We have previously engineered MSC-EVs to encapsulate a photosensitizer (mTHPC) for improved photodynamic therapy, efficiency and safety (data will be published in 2020). In mouse model of colorectal PC, we have demonstrated a highlighted and superior biodistribution of mTHPC in the tumor with EVs-mTHPC compared to other mTHPC formulations (free and liposomal (the tumour/normal tissue ratios were 44 vs 1.4 and 5.7 respectively). We have shown a therapeutic efficiency in terms of tumoral cytotoxicity (72.5% of tumors presented necrosis after PDT, compared to 13% after control treatment,

p<0.0001), immunomodulation (the tumoral infiltration of various inflammatory cells was more important with PDT after EVs-mTHPC injection than others groups (mean $CD8^+$ T-cell : 4.4% vs 1.1%, p<0.0001 ; and mean F4 80+: 41% vs 20.4%, p<0.0001 ; respectively), and survival time (median survival time was 28,22 and 16 days after EVs-mTHPC+PDT, control or free-mTHPC+PDT treatment respectively, p<0.0001). EVs nanovectorization permitted safety for surrounding tissue after laser irradiation (we observed liver and tubular necrosis without nanovectorization). Taking together, all these results demonstrated that in an animal model of colon cancer PC, using VEs can offer a new solution to bio-target drug delivery, using an intraperitoneal approach.

In addition, preliminary data reported an immune activity of EVs (without drug), (in comparison with no treatment), with an intra-tumoral decrease of M2 macrophages (exhibit pro-tumoral activity) (3.71% ± 0.44 vs 5.96% ± 0.61 respectively, p=0.002) for an equivalent percent of total macrophages (F4 80 : 23.26% ± 2.5 vs 19.34% ± 2.6, p=0.13), and an increase of T lymphocytes CD3 (2.55% ± 0.3 vs 0.71% ± 0.1 respectively, p=0.0001).

We postulated that MCS-EVs could allow a double anti-tumoral action : drug nanovectorization and M2 reprogramming towards an M1 phenotype. We expect to demonstrate a tumoral drug biodistribution around 50 times more important with biological vectorization, allowing a major decrease of iatrogenic effects (including hepatic, renal and cardiac toxicity of anticancer drugs) and a major/significant tumoral regression. The combination of therapeutics classes could allow accumulation of therapeutic effects, but without toxicity.

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