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# The significance of the omentum in locoregional immunotherapy for peritoneal carcinomatosis

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**ABSTRACT**

Our recent research has unveiled the potential of locoregional immunotherapy. Cytokine-armored viral vectors, such as modified vaccinia virus Ankara vector encoding single-chain interleukin-12 (MVA.scIL-12), can target the omentum and elicit a robust tumor-specific immune response, all the while minimizing toxicity.

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Peritoneal carcinomatosis (PCa) represents an advanced stage of gynecological and gastrointestinal malignancies, with the highest incidence occurring in ovarian and colorectal cancer patients. In PCa, cancer cells exfoliated from primary lesions disseminate throughout the peritoneal cavity, leading to the formation of metastatic nodules on peritoneal surfaces.[1] Among the different tissues within the peritoneum, the omentum is notably significant in the progression of PCa. The omentum provides an ideal environment for initial tumor cell nesting, likely due to its rich vascularization, fat deposits, and role in tissue regeneration.[2] Interestingly, the omentum is also abundant in fat-associated lymphoid clusters (FALCs), historically referred to as "*milky spots*", which serve as the first line of immunological defense against pathogens reaching the peritoneal cavity.[3] Paradoxically, this local immune response appears to be subverted by tumor cells and immune modulators present in the context of peritoneal metastasis, ultimately contributing to disease progression and poor patient outcomes. Collectively, these characteristics render the omentum an attractive target for locoregional cancer immunotherapy strategies. These strategies can enhance the immune response against malignant cells within the peritoneal cavity while minimizing potential systemic side effects.

In 2016, Katz, *et al.*, presented compelling evidence in favor of the intraperitoneal route over systemic administration in the context of CAR-T cell therapy targeting the CEA antigen in mouse models of colorectal PCa. When compared to systemic therapy, intraperitoneal administration demonstrated a more robust and prolonged response against $CEA^+$ peritoneal tumors. This approach showcased an increase in the effector memory phenotype over time in the CAR-T cells that were injected. Additionally, it provided protection against extra-abdominal lesions, suggesting that intraperitoneal CAR-T therapy might mediate a phenomenon similar to the abscopal effect as infrequently described following radiation therapy.[4,5] Besides CEA, intraperitoneal administration of other CAR therapies targeting various tumor epitopes, such as MUC-16, HER-2, and FRα, is under evaluation in both preclinical and clinical settings (NCT03585764, NCT03907527, NCT04684459). Furthermore, the infusion of oncolytic virus-based immunotherapies (*e.g.*, GL-ONC1 and T-VEC) into the abdominal cavity via an implanted peritoneal catheter is being assessed for the treatment of advanced PCa, with positive clinical results reported (NCT01443260, NCT02759588, NCT03663712).[6]

Our research team had previously demonstrated that the intraperitoneal administration of OVA-specific OT-I T cells electroporated with IL-12 mRNA led to their rapid entry into the omentum. This approach elicited a more potent antitumor response as compared to the systemic route.[7] Furthermore, we had reported that the intraperitoneal injection of a recombinant MVA vector (rMVA) encoding tumor-associated antigens, in conjunction with the co-expression of CD40L and CD137L, two potent immunostimulatory molecules with complementary mechanisms of action, resulted in a robust immune-mediated antitumor response associated with improved survival in aggressive mouse models of PCa.[8] Despite these promising results, the precise mechanisms underlying the superior anti-tumor immune response elicited by the intraperitoneal route remain poorly defined.

In our most recent publication entitled, "Intraperitoneal Administration of a Modified Vaccinia Virus Ankara Confers

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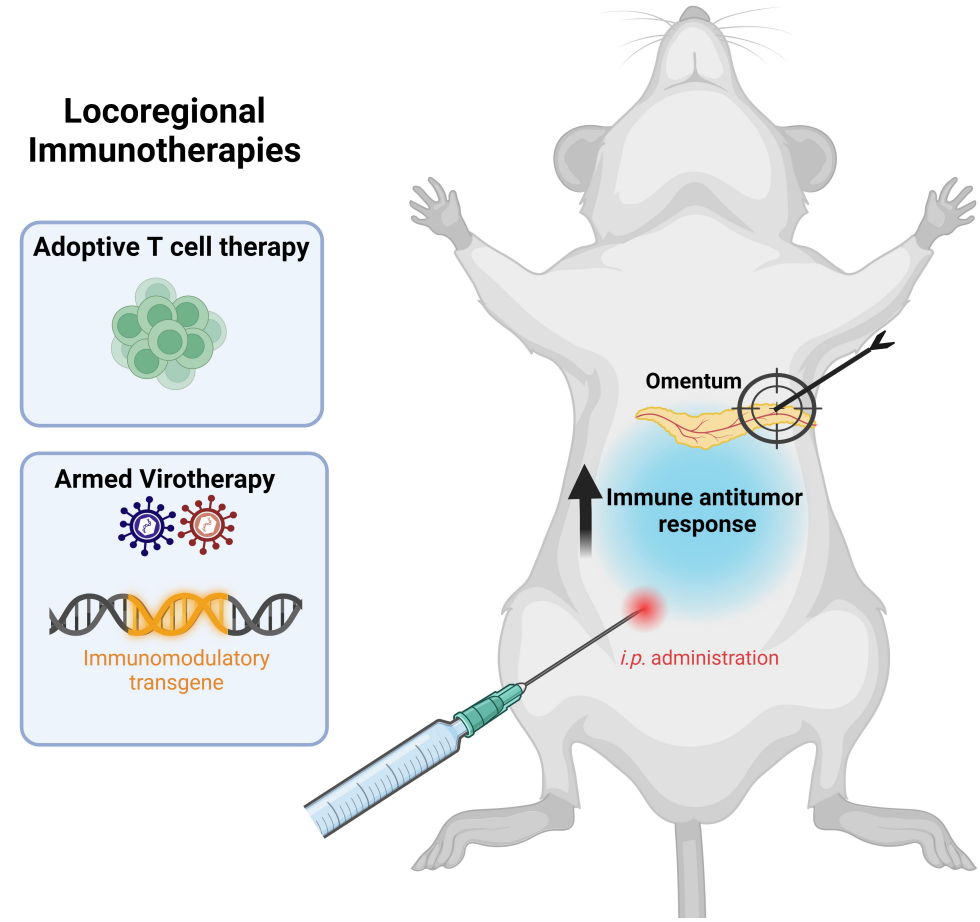


**Figure 1.** The omentum is a "*key player*" in the efficacy of locoregional immunotherapies against peritoneal metastasis. Peritoneal carcinomatosis immunotherapies can be given locally into the peritoneal cavity including adoptive T cell therapy and virotherapy with genetically engineered viral vectors expressing immunomodulatory molecules. In mouse models of PCa, these immunotherapy strategies have been proven to target the omentum and elicit a robust antitumor-specific immune response while minimizing the toxicity associated with systemic delivery.

Single-Chain Interleukin-12 Expression to the Omentum and Achieves Immune-Mediated Efficacy Against Peritoneal Carcinomatosis",[9] we presented, for the first time, the selective targeting of a viral vector-based immunotherapy to the omentum following intraperitoneal administration. Expression levels were found to be 10 times higher in the omentum as compared to other organs within the peritoneal cavity, such as the spleen and mesentery. Furthermore, we conducted an investigation into the expression of the transgene (IL-12) within the tumor-bearing omentum using flow cytometry. IL-12 was detected in both hematolymphoid ($CD45^+$) cells and nonimmune ($CD45^-$) cell populations, including tumor cells. We also provided an in-depth analysis of how the local expression of the proinflammatory cytokine IL-12 in the omentum impacted the transcriptomic profile of infiltrating T lymphocytes. A comparison between different routes of vector administration (intraperitoneal and intravenous) revealed the induction of several pathways related to cellular metabolism that were up-regulated and indicated a decrease in the presence of macrophages and B cells. Moreover, through the use of intravital confocal microscopy, we were able to determine that the intraperitoneal administration of MVA.scIL-12 significantly enhanced the infiltration of CD8 T cells into omental tumor nodules as shortly as 24 hours after treatment. Additionally, the percentage of T cells in contact with tumor cells was higher, indicating improved performance of the immune cells as a result of treatment.

In terms of the elicited immune response and tumor control, intraperitoneal administration of MVA.scIL-12 proved to be more effective than intravenous administration and effectively mitigated the toxicity associated with systemic scIL-12 administration. Furthermore, intraperitoneal administration exhibited control over both peritoneal and subcutaneous tumors. In contrast, intratumor administration only managed to control the progression of the treated subcutaneous lesions, underscoring the potency of the intraperitoneal route in the generation of more robust local and systemic immune memory responses.

To further investigate the role of the omentum in the previously described antitumor activity, we conducted omentectomy, the surgical removal of the omentum. Four weeks later, we evaluated the intraperitoneal treatment with MVA.scIL-12 in an aggressive model of PCa using MC38 cells. Our results revealed a significant reduction in the survival of mice in the omentectomy group. In alignment with these findings, a recent publication by David A. Christian, *et al.*, demonstrated that omental resident cDC1 cells play a crucial role as a result of providing

secondary signals necessary for the expansion and memory differentiation of CD8 T cells in response to intraperitoneal microbial challenges.[10]

Collectively, these findings provide compelling evidence regarding the significance of the omentum in orchestrating a robust immune response. This has implications when considering the appropriateness of omentectomy in patients with PCa, especially given the growing number of clinical trials assessing various immunotherapy approaches in this context. Additionally, these results underscore the necessity for more comprehensive studies on the molecular mechanisms responsible for the superior performance of the intraperitoneal route, particularly in the context of PCa.

In conclusion, intraperitoneal immunotherapy with viral vectors demonstrates the potential for the treatment of peritoneal metastasis, and the available evidence emphasizes the critical role of the omentum in generating a potent and effective immune antitumor response (Figure 1). Although challenges remain, locoregional or intracavitary immunotherapies offer hope to patients facing this devastating stage of their disease.

## Abbreviations

| | |
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| PCa | peritoneal carcinomatosis |
| rMVA | recombinant MVA vector |
| scIL-12 | single chain Interleukin 12 |

## Disclosure statement

P.B. and F.A. received research funding from Bavarian Nordic. I.M. reports receiving commercial research grants from AstraZeneca, BMS, Highlight Therapeutics, Alligator, Pfizer Genmab and Roche; has received speakers bureau honoraria from MSD; and is a consultant or advisory board member for BMS, Roche, AstraZeneca, Genmab, Pharmamar, F-Star, Bioncotech, Bayer, Numab, Pieris, Gossamer, Alligator and Merck Serono. A.B. has no conflict of interest to declare.

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