Controlling treatment toxicity in ovarian cancer to prime the patient for tumor extinction therapy

High-grade serous ovarian cancer (HGSOC) remains the most lethal gynecologic malignancy, taking nearly 13000 lives annually. Although therapeutic advances involving PARP-inhibitors have benefited a subset of homologous recombination (HR)-deficient HGSOC patients, the remaining HR-proficient groups have seen no improvement in long-term survival. In addition to the limited therapeutic responses, toxicity remains a major therapeutic limitation. Thus, *there is an urgent need for the development of effective strategies to predict and implement non-toxic and effective treatments that could lead to cure for HGSOC patients, which requires that we recognize, predict, pre-empt, and remediate toxicity*.

The composition of the gut microbiome and its products, the meta-metabolome or microbiome, has emerged as a central mediator of anti-tumor immunity in HGSOC. Toxicity to the gut microbiome, "metatoxicity," is ubiquitous during cancer therapy, yet because metatoxicity is largely unrecognized, it has until now remained unaddressed, even though remediation could be simple and inexpensive. An important mediator of gut bacterial regulated anti-tumor effects is the production of short-chain fatty acids (**SCFA**), which interact with immune and tumor cells, and positively associate with treatment response. Conversely, long-chain fatty acids (**LCFA**) promote immune evasion and tumor growth in part by intrinsic activation of the endoplasmic reticulum stress driver **PERK** and promotion of epigenetic changes. Microbiome-related SCFA/LCFA ratio is drastically diminished by carboplatin + paclitaxel chemotherapy, and also by PARP-inhibitor therapy, and does not recover without intervention. Thus, using mathematical model-informed combination strategies, we propose that lower SCFA/LCFA ratio, chromatin methylation alterations, and PERK activation drives toxicity and decreases effectiveness of subsequent rounds of chemotherapy, targeted therapies (PARPi), and even advanced therapies such as CAR-T cell immunotherapy. This also represents an opportunity for interventional approaches to reduce toxicity and boost therapeutic effects.

Our *long-term goal* is to create innovative strategies that effectively predict, prevent, and overcome toxicity in HGSOC patients. Our *overarching hypothesis* is that using mathematical modelling to predict and control direct and indirect toxicity will enable us to precisely combine, sequence, and administer the optimal volume of "second strike" therapies, increasing chances for HGSOC cure. This is supported by encouraging mathematical modelling preliminary results indicating the effects of lower SCFA/LCFA ratio and increased activation of PERK as activators of toxicity and limitations to therapeutics. Thus, development of the proposed mathematical informed models will enable us to predict and buffer direct and indirect toxicity in HGSOC patients, while testing interventional strategies to overcome toxicity and support the development of protective immunity for the benefit of CAR-T immunotherapy. We will test the hypothesis through the following *Specific Aims*:

Aim 1. To anticipate and pre-empt treatment-related toxicity risk using a machine learning classifier based on PBMC fluctuating methylation clocks. We *hypothesize* that low diversity of peripheral blood cell subsets is a novel biomarker of poor bone marrow health and can identify patients at high-risk of toxicity, informing when to switch or pause therapy. We will develop a machine learning classifier to predict toxicity risk from PBMC composition, methylation clocks, and patient baseline characteristics. We will train our classifier on publicly available data and validate it using methylation arrays from blood samples from 30 patients at 3 time points during first-line chemotherapy (available under MCC 21244).

Aim 2. To buffer the direct toxicity of PARPi-targeted therapy combinations in HGSOC using dosing optimized via mathematical modelling. We *hypothesize* that a sequence of targeted therapy combinations following frontline platinum-based chemotherapy optimized with mathematical modeling will minimize toxicity and improve efficacy. We propose to use patient-derived models of HR-proficient HGSOC to validate promising sequential PARPi targeted therapy combinations identified by mathematical modeling of cell cycle dynamics and PERK activation. We will focus on drugs already in clinical development including PARP, ATR, and PERK inhibitors, which will allow for rapid clinical translation.

Aim 3. To mitigate indirect toxicity induced by carboplatin-paclitaxel chemotherapy on the gut microbiome to prime the patient for curative intent immunotherapy. We *propose* that educated use of SCFA in the form of butyrate will buffer toxicity changes induced by carboplatin+taxol chemotherapy, and also by PARP-inhibitor, thereby increasing effectiveness and synergizing with CAR-T cell therapy. We will validate mathematical modeling of microbiome dynamics using patient samples (under MCC 21244).

Overall impact: We will use for the first-time mathematical models to link microbial SCFA, methylation changes, and sustained ER stress as a process that regulates toxicity and therapeutic activity. Also, measurement and remediation of metatoxicity could enable to strategically sequence, combine, and pause therapies. Finally, outlined pharmacological combinations will pave the way to boost the effects of CAR-T cells in HGSOC.