injected subcutaneously or orthotopically in syngeneic mouse models according to cell-passage number. Moreover, we found that MMR-driven dynamic generation of neoantigens, when restricted to a clonal population, further increases immune detection. Mechanistically, MLH1 inactivation increased the mutational burden and led to dynamic mutational profiles, resulting in persistent renewal of neoantigens *in vitro* and *in vivo*, while control cells exhibited stable mutational loads and neoantigen profiles over time. These results led us hypothesize that enforced increase of the number of mutations in cancer cells could restrict cancer growth and might be beneficial for therapeutic purposes. We therefore performed a pharmacological screen to identify agents capable of permanent inactivation of MMR in colorectal, breast and PDAC cancer cells. We found that temozolomide triggers MLH1 inactivation in cancer cells that -as a result- are unable to form tumors in syngeneic animals. Genomic analysis of temozolomide resistant cells revealed that fluctuating levels of neoantigens, rather than the absolute number of mutations, might be critical to provoke immune surveillance. Overall, these results provide the rationale for developing innovative anticancer therapies based on inhibition of DNA repair mechanisms.

**SP-0454 Trial design: early clinical studies and learning from the laboratory**

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**Abstract text**

Radiotherapy (RT) is a highly effective cancer treatment and research for much of the last century focused on understanding the mechanisms underlying its tumouricidal properties. Only relatively recently have the local and systemic immunomodulatory properties of RT been investigated. RT is now known to stimulate an immune response by inducing a variety of immunogenic and phenotypic changes in malignant cells that can recalibrate the immune contexture of the tumour microenvironment. If the immunoregulatory effects could be harnessed this could significantly increase the anti-cancer effect of RT The recent major breakthrough of the immune checkpoint inhibitors (ICI) anti-CTLA-4 (cytotoxic T-Lymphocyte-associated protein 4) and anti-PD1/PD-L1 (programmed death-ligand 1) has led to durable remissions and improved survival in a number of incurable cancers including metastatic malignant melanoma, Non Small Cell lung cancer, renal cell cancer and other cancers. This remarkable clinical efficacy has established immunotherapy as another effective form of cancer therapy and stimulated the "immunotherapy revolution" leading to the large scale development of a new class of therapeutics termed immuno-oncology (IO) agents. Given that both RT and IO agents are potentially immunomodulatory combining RT with IO agents provides a unique opportunity to increase systemic anti-tumour immunity and transform cancer therapy. This goal would be achieved with RT and IO agents combinations by improving the outcomes for those patients who present with localised disease and who currently fail with localised recurrence or the development of metastatic disease as well as tackling those with oligometastastic disease who are currently incurable and thus converting RT into an effective part of systemic therapy. Proof of principle preclinical studies using RT in combination with Immune check point inhibition (ICI) using anti-CTLA-4 and PD-1/PD-L1 blockade have demonstrated "abscopal" systemic anti-tumour immune responses in tumours outside of the RT field leading to long term clearance. These systemic immune responses have also been reported in a number of clinical case reports. This preclinical evidence and these interesting clinical reports led to hundreds of clinical trials being launched with the aim of testing the efficacy of radiotherapy in combination with immunotherapy to improve outcomes. However converting clinical responses seen with in a minority of patients in solid tumours with ICI using a single site of RT for "abscopal" responses and expecting he majority of patients to respond to ICI in combination with RT is unlikely to happen. In order to see more durable responses to RT and IO agent combinations we need firstly to further understand how RT influences the tumour cells, tumour microenvironment and surrounding immune effector cells. We also need to explore irradiation of multiple lesions in order to enhance the likelihood of obtaining meaningful clinical outcomes in some clinical situations. Secondly we need to partner RT with the optimal immunomodulatory IO agent to generate the most effective systemic anti-tumour immune responses. For tumours rich in T cell infiltrates, RT and anti-PD1 might be effective combinations overcoming T cell exhaustion and increasing systemic T cell anti-tumour immunity. For those with more immunosuppressive microenvironments that might be devoid of T cell and / or rich in myeloid cells other types of immunostimulatory agents are likely to be required. In this presentation the current challenges in translating recent findings with RT and IO agents in the laboratory into the clinic and a review of the progress made to date in clinical trials will be discussed.

## Symposium: Tumor Metabolism and Radiotherapy

**SP-0455 Inhibition of glycolysis and redox metabolic pathways in cervical cancer**

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**Abstract text**

One third of locally advanced cervical cancers treated with standard of care chemoradiation (pelvic irradiation plus concurrent cisplatin chemotherapy) fail this treatment, and there is currently no cure for recurrent or metastatic disease. Our previous work has shown that the results of $^{18}$F-fluoro-deoxy-glucose positron-emission-tomography (FDG-PET) can be used to identify treatment-resistant cervical cancers. Cervical tumors that take up large quantities of FDG, a glucose analog, prior to treatment are resistant to radiation, and tumors that maintain high levels of FDG uptake after radiation is complete are likely to recur. Given these clinical observations, a reasonable hypothesis is that inhibiting cervical tumor glucose metabolism may improve radiation sensitivity. The hexokinase inhibitor, 2-deoxyglucose (2-DG), has been evaluated in *in vitro* and *in vivo* as a radio-sensitizer in a number of cancer models, and 2-DG has been administered to patients in the context of clinical trials. The results of these studies show that while inhibition of glucose metabolism with 2-DG can transiently increase radiation sensitivity, long term therapeutic gains are limited by the normal tissue toxicity that occurs at the doses required to maintain effective inhibition of glycolysis in tumors. In addition, new concerns have arisen over the capacity of tumor cells to rewire their metabolism in response to metabolic therapy (metabolic plasticity). Glycolytic tumor cells exist in a relative state of oxidative stress due increased levels of reactive oxygen species (ROS) and compensate for this by upregulating redox metabolic pathways. Indirect effects of irradiation also result in transient increases in ROS that are metabolized by tumor cells. We hypothesized that simultaneous inhibition of glycolysis and the redox metabolic pathways would be more effective as a radiation sensitizer than inhibition of glycolysis alone. Using a panel of cervical cancer cell lines, we