

**TITLE: Statistical Analysis of Enhanced CTL killing Activity Against Irradiated Tumor Cells**

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**Background:** Radiation is the most commonly used cancer treatment and has recently been shown to enhance the effectiveness of immune-based therapies. The most effective immunotherapy strategies generate tumor-associated antigen (TAA) specific cytotoxic T-cells (CTLs) capable of killing tumor cells. We have previously shown enhanced killing of colorectal carcinoma cells by tumor-specific CD8+ CTLs following sub-lethal tumor irradiation in vitro. Tumor cells, however, can induce a variety of immunosuppressive mechanisms, including the induction of CD4+ regulatory T cells (TREGS). TREGS are capable of directly inhibiting anti-tumor attack by CTLs, resulting in tumor escape from immune elimination. More recently we have seen changes in the expression of genes that are known to alter the function of both CTLs and TREGS. In addition, we have a funded grant to explore the impact of irradiated tumor cells on TREG function. Identifying therapies that could reverse induction of TREG cells is essential to improving immunotherapy strategies. The goal of this study is to determine if gene expression patterns exist that correlate with increased CTL activity and decreased TREGS function. We hypothesize that exposure of tumor cells to sub-lethal doses of IR will induce a molecular signature of enhanced immunogenicity that results in decreased TREG activity and increased CTL activity. A major objective of this study is to identify a set of radiation-induced molecules in tumor cells that correlate with altered immune cell activity.

**Methods:** Changes in gene expression will be evaluated using standard flow cytometry (for protein) and q-RT-PCR (for mRNA) methods. Experiments will help identify a set of candidate molecular markers that can discriminate between cancer cells killed by CTLs and those that are not following IR. To achieve this we will investigate if there is a relationship between immune cell function (CTL killing of tumor cells or TREG cell suppressive activity) and a gene expression pattern. To determine concordance among cell lines post-IR, protein and mRNA expression signatures will be analyzed using linear regression. For this analysis, gene expression will be transformed into fold-change of irradiated (10Gy or 5Gy) versus 0Gy and normalized to input cell numbers. Immune cell function will also be reported as fold-change to describe increases in CTL activity (and decreases in TREG activity) following tumor irradiation

**Results:** We analyzed data from the following cell cultures: Colo205, HCT116, and SW620 that were in the following irradiation conditions: 0Gy, 5Gy and 10Gy. We first determined the changes from baseline in the higher irradiation conditions, that is, we computed differences in the gene expression from 0Gy to 5Gy, and from 0Gy to 10Gy. A treatment is deemed to be significant if the slope is significantly different from 0. For Colo205 cells, we have obtained a value of  $\beta = 0.365$  for the slope, corresponding to a p-value of 0.718; we conclude that this effect is not statistically significant. For the HCT116 cells, we obtained  $\beta = 1.555$  and  $p = 0.139$ . For the SW620 cells we obtained  $\beta = 1.777$  and  $p = 0.090$ . The data for the HCT116 and SW620 cells is fairly close to being statistically significant at  $\alpha = 0.1$ .

**Future directions:** We plan to collect additional data points for these cell lines in order to validate our results at  $\alpha = 0.05$ . Results of the proposed research will expand information available regarding immune relevant molecular changes induced in carcinoma cells following IR and facilitate progress towards achieving the long-term goal of elucidating the mechanistic link between IR and increased tumor attack by CTLs.