TGFβRI Inhibition Prior to Radiation Enhances Efficacy in a CD8 Dependent Fashion

K. Young[1], P. Newell[2,3], B. Cottam[2], D. Friedman[2], T. Savage[2], J. R. Baird[2], E. Akporiaye[2], M. J. Gough[2], M. Crittenden[2,3],

[1]Oregon Health & Science University, Portland, OR, [2]Earle A. Chiles Research Institute, Providence Portland Medical Center, Portland, OR, [3]The Oregon Clinic, Portland, OR

Purpose/Objective(s): The immune infiltrate in colorectal cancer has been correlated with outcome, such that individuals with higher infiltrations of T cells have increased survival independent of disease stage. For those patients with poor immune infiltrates, overall survival is severely limited. Since the colorectal cancer patients studied received conventional cancer therapies, these data could be interpreted to mean that the pre-treatment tumor environment increases the efficacy of treatments such as chemotherapy, surgical resection and radiation therapy. This study was designed to test the hypothesis that an improved immune environment in the tumor at the time of treatment will increase the efficacy of radiation therapy.

Materials/Methods: Balb/c mice were challenged with either CT26 colorectal or Panc02 pancreatic tumor cells and seven days later treated with mouse chow containing control or a TGFβ type I inhibitor, SM16, for one week followed by tumor-only high-dose radiation. Survival, tumor growth, and tumor immune cell infiltrate were analyzed.

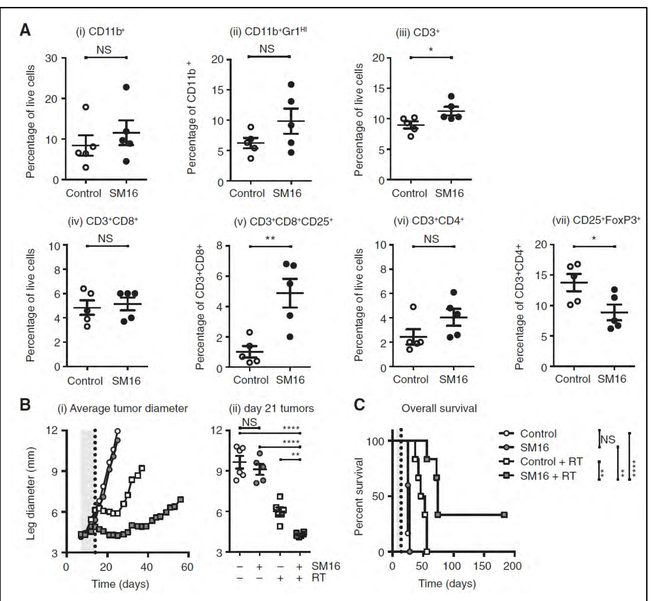

Figure 1. SM16 alters the tumor immune infiltrate and improves survival in combination with radiation. BALB/c mice bearing d7 CT26 tumors were treated with control or SM16 chow for 7d. a) Flow cytometry of tumor infiltrating cells on day 14. b) i) Average diameter of CT26 tumors treated with control chow or SM16 chow. On day 14, mice received 20Gy of radiation. ii) individual tumor diameters at day 21, 7 days following radiation therapy and cessation of SM16 treatment. c) Survival of mice treated as in b). Median survival: Control 25d; SM16 28d; RT 49.5d; RT+SM16 74d. NS = not significant; * = p<0.05; ** =p<0.01; *** =p<0.005; **** =p<0.001.

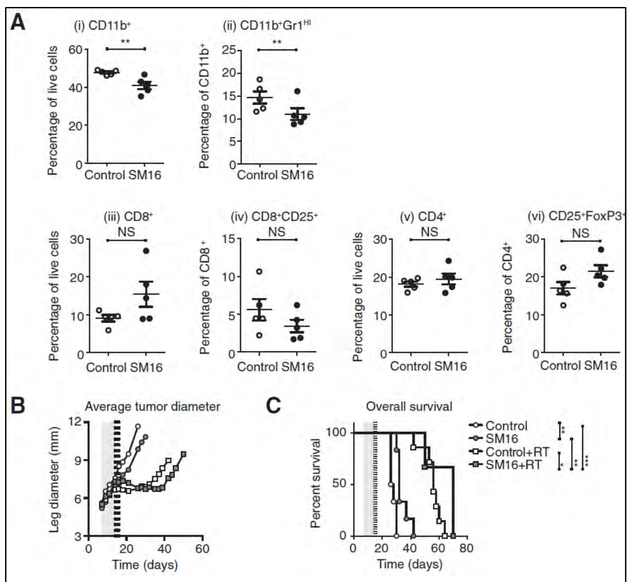

Figure 2. SM16 treatment in Panc02 tumors alters immune infiltrate and improves survival. C57BL/6 mice bearing Panc02 tumors were treated on d7 with control or SM16 chow for one week. a) Flow cytometry of tumor infiltrating cells on day 14. b) Mice bearing Panc02 tumors were treated with control or SM16 chow as above. On day 14-16, tumors were irradiated with 20Gy daily for three consecutive days. c) Overall survival of mice treated as in b). Median survival: Control 27d; SM16 32d; RT 56d; RT+SM16 70d. NS = not significant; * = p<0.05; ** = p<0.01; ***=p<0.005. Statistics calculated by t-test – infiltrates, Logrank – survival.

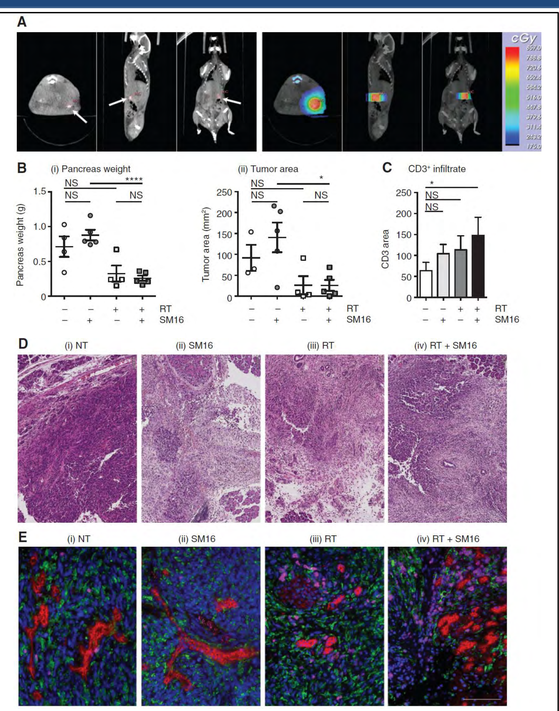

Figure 3. Orthotopic pancreatic adenocarcinomas demonstrate treatment effect of radiation and SM16. a) Cone beam CT scan of C57BL/6 mice bearing orthotopic Panc02 tumors with lipiodol for CT localization (white arrow). 8Gy radiation was delivered using a 180 degree arc treatment and a 5x 5mm collimator, with dose cloud as demonstrated. b) Mice were treated with control or SM16 chow for one week beginning 7 days after tumor challenge. 8Gy radiation delivered on d14 and every other day for 3 treatments. Tumor size was determined by i) weight of excised pancreas and ii) cross-sectional area via histology. c) Quantitation of CD3 cell infiltrate from immunofluorescence images. d) H&E staining of tumors given i) control chow, ii) SM16 chow, iii) control chow plus 8Gy x 3 fractions, or iv) SM16 chow plus 8Gy x 3 fractions. e) Immunofluorescence for E-cadherin (red), F4/80 (green), CD3 (magenta), and DAPI nuclear staining (blue) in tumors treated as per c). Scale bar 50μm. NS = not significant; * = p<0.05; ****p<0.0001.

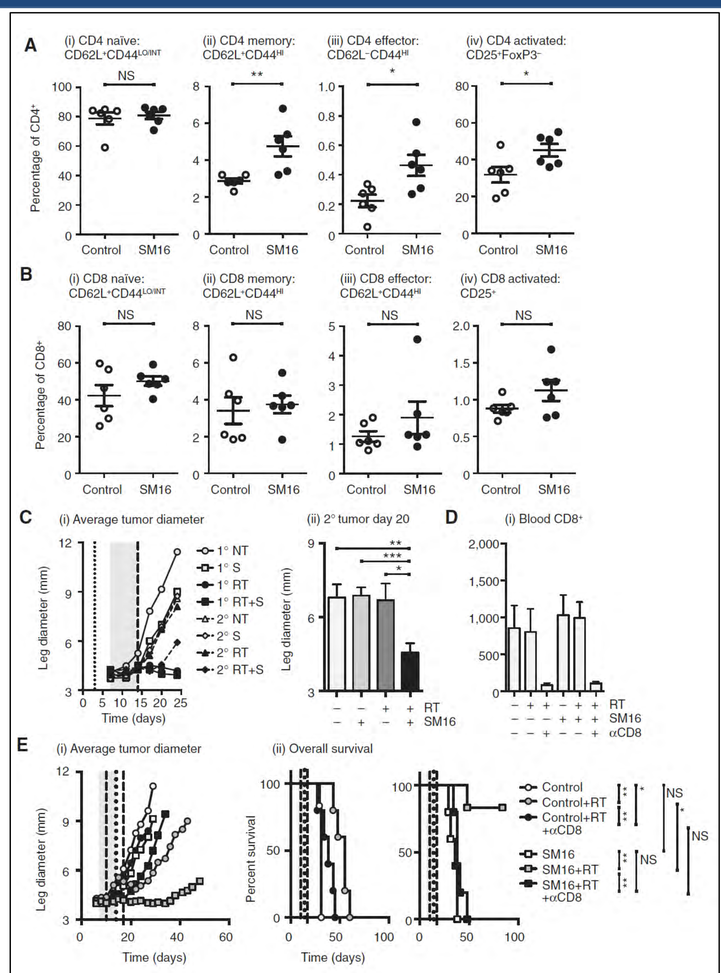

Figure 4. The increased efficacy of radiation with SM16 is dependent on CD8 T cells. Mice bearing CT26 tumors, were fed control or SM16 chow for one week beginning on day 7 and treated with 20Gy radiation on day 14. Tumor draining lymph nodes were harvested on day 15 and a) CD4 and b) CD8 T cells analyzed via flow cytometry for i) naïve, ii) memory, iii) effector and iv) activated subpopulations. c) Concomitant CT26 tumors were established in bilateral hindlimbs and treated with control or SM16 chow for one week followed by 20Gy of radiation to the right hindlimb tumor on day 14. i) average tumor diameter and ii) the untreated left hindlimb tumors on day 20. d) CT26 tumor-bearing mice were treated with control or SM16 chow as above. Anti-CD8 antibody was injected intraperitoneally on day 10, 17, and 24. Quantitative flow cytometry of peripheral blood for i) CD8 T cells and ii) CD4 T cells to confirm selective depletion. e) i) Average tumor size for mice treated as in c), with control or SM16 chow as above. Day 14 tumor radiation (dotted line) and CD8 depletion (dashed lines). At d22, 8 days following RT mean tumor area of treated tumors is significantly smaller than untreated (Control p<0.001, SM16 p<0.01) but not where CD8 depleted. ii) Survival of mice treated as in i) with control chow treated mice depicted in the graph on the left and SM16 treated mice on the right. Median survival: Control 31d; Control RT 55d; Control+RT+anti-CD8 38d; SM16 36d; SM16+RT undefined; SM16+RT+anti-CD8 38d. NS = not significant; * = p<0.05; ** = p<0.01.

Results: We demonstrate that inhibition of TGFβ using the orally available small molecule inhibitor SM16 improved the immune environment of tumors in mice by increasing T cell infiltrate and decreasing inhibitory immune cell infiltrate including T regulatory cells and myeloid derived suppressor cells (p<0.05); and significantly improved the efficacy of subsequent radiation therapy in colorectal (median survival 74 days versus 49.5 days, p< 0.01) and pancreatic tumor models (median survival 70 days versus 56 days, p<0.05). This effect was not due to changes in radiosensitivity, epithelial to mesenchymal transition or changes in vascular function in the tumor; rather, this effect was entirely dependent on adaptive immunity and resulted in distant tumor responses (p<0.05) and long-term protective immunity in cured mice.

Table 1. Long-term tumor-specific protection in cured mice

Tumor	4T1	CT26
Tumor+ mice	7	0
Mice challenged	7	7

NOTE: Mice cured of CT26 tumors by SM16 pretreatment followed by radiotherapy were rechallenged on opposing flanks with 4T1 and CT26 cells. Mice were followed for development of palpable tumors at the injection site. Table shows the number of mice developing palpable tumors (Tumor+) as compared with the number of mice challenged.

Conclusions: These data demonstrate that immunotherapy is an option to improve the immune status of patients with poor tumor infiltrates and that pre-treatment improves the efficacy of radiation therapy.

Future Directions: 1) Further characterize the mechanism of improved radiation efficacy.
2) Translate into the clinic for locally advanced rectal adenocarcinoma patients receiving neoadjuvant chemoradiation.

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