

Tumor immune remodeling by TGF β inhibition improves the efficacy of radiation therapy

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The tumor immune environment has been linked to prognosis in patients with a range of malignancies. Recently, we demonstrated in pre-clinical models that modifying the tumor immune environment using a small-molecule inhibitor of TGF β significantly improved outcome to subsequent radiation therapy. These data suggest that this and other immunotherapies may be used to remodel the tumor before conventional cancer therapies to improve outcomes.

Recent studies have explored the link between tumor immune cell infiltrate and overall survival, reporting that decreased T cell infiltrate and increased macrophage infiltrate correlate with decreased survival.^{1,2} These data are particularly dramatic in colorectal cancer, where there is an international effort underway to evaluate immune infiltrates, or “immune score” in tumors as a prognostic tool for patients.¹ For those patients identified with poor immune scores, the question remains as to whether the tumor immune status can be improved, and whether that will increase the efficacy of treatment.

We hypothesized that an improved immune environment at the time of treatment would increase the efficacy of radiation (Fig. 1). To improve the immune environment of established tumors, we used targeted inhibition of TGF β . During carcinogenesis, TGF β can act as a tumor suppressor; though once invasive carcinoma has developed, TGF β is an important force that sustains the invasive and immune suppressive phenotype of tumors. The small molecule Alk5 inhibitor SM16 is readily bioavailable and chronic administration of dietary SM16 re-polarizes tumor-infiltrating myeloid cells,^{3,4} and dramatically improves the function of T cell targeted immunotherapy.³ Our experiments demonstrate that pretreatment with SM16 improved the immune environment of tumors in mice, and significantly

improved the efficacy of subsequent radiation therapy.⁵ We demonstrated that this effect was entirely dependent on CD8⁺ T cells and generated long-term tumor-specific protection.⁵ In addition, using a concomitant tumor model we found that SM16 administration followed by radiation to one tumor resulted in growth delay of distant tumors that was not seen with either modality alone.

The mechanisms why improving the pre-treatment tumor immune environment permits CD8⁺ T cell control of tumors remains to be determined. This may relate to an improved context of antigen presentation following radiation-induced antigen release, or since we see fewer T regulatory cells in the tumor, this may relate to decreased T regulatory cell-mediated suppression of immune responses that are initiated by radiation

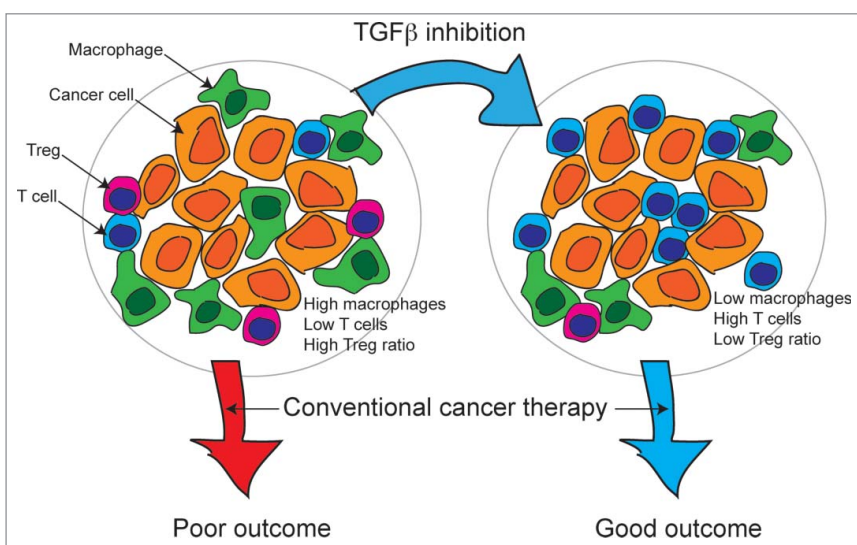


Figure 1. Immunotherapy to improve pre-treatment tumor immune environment to improve outcome with conventional cancer therapy. Patients bearing tumors with high macrophage infiltrates, low T cells infiltrates and high Treg:CD8⁺ ratios have been shown to have poor prognosis when treated with conventional therapies. Immunotherapy can change the infiltrates in tumors, and our studies with TGF β inhibition suggest this can improve outcome.

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therapy. Since TGFβ suppresses the effector function of T cells in tumors,^{3,6} it is likely that TGFβ inhibition improves the effector phase, though in our studies the short *in vivo* half-life of SM16 means that inhibition is not sustained through any effector phase initiated by radiation therapy. Similarly, while myeloid responses to radiation therapy may suppress adaptive immune responses at later times following radiation,⁷ these later time points will not be affected by pretreatment with SM16. Since we see SM16-mediated early improvements in CD4⁺ differentiation in the tumor draining lymph node at early time points following radiation, we suspect that TGFβ inhibition permits CD4⁺ T cells to differentiate toward more helpful

phenotypes that support rather than suppress effector CD8⁺ function.

TGFβ inhibition combined with radiation has a great deal of potential for many other reasons. TGFβ inhibition has been shown to reduce the severity of lung fibrosis⁸ and rectal fibrosis⁹ in murine models. In addition, TGFβ inhibition at the time of radiation increases radiosensitivity *in vitro* and *in vivo*.¹⁰ In our studies, we did not observe changes in epithelial-to-mesenchymal transition in the tumor or changes to tumor vasculature,⁵ perhaps due to the short duration of inhibition or due to the Alk5 specificity of the inhibitor. However, it may also be prudent to be cautious of extended TGFβ inhibition in the setting of radiation therapy, particularly in patients who have a "field

cancerization effect," since there is potential for second malignancy.

Based on these studies, we conclude that there are significant opportunities to manipulate the immune environment of tumors for therapeutic gain. Since the immune environment of colorectal tumors is so important to outcome through conventional cancer therapies,¹ it is heartening to see that there is potential to improve outcomes for those with poor immune infiltrates through upfront immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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