Toca 511 and 5-FC induces T cell-mediated antitumor immunity in a mouse glioma model which is enhanced by the addition of a therapeutic antibody against CTLA-4 and correlative with a reduction in memory T regulatory cells.

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Abstract

Toca 511 (oncophage antineoplastic) is a gamma retrovirus replicating vector that selectively infects cancer cells in vivo and encodes cytokine downstream. In combination with the prodrug, 5-fluorocytosine (5-FC), Toca 511 produces 5-fluorouracil (5-FU) locally in the tumor microenvironment. Prior work has demonstrated a reduction in immunosuppressive myeloid cells and an increase in CD8 and CD10 T cells in tumors while T regulatory cells remain unchanged in comparison with Toca 511 and 5-FC. This work, in a mouse model of glioma, aimed to determine if the addition of a checkpoint inhibitor, αCTLA-4, would provide therapeutic benefit to Toca 511 and 5-FC. Initially, we noted that Toca 511 and 5-FC was highly efficacious and that it provided little room for further improvement and therefore combination with αCTLA-4 did not show additive benefit against the primary cancer. However tumor associated Regulatory T cells were significantly reduced with αCTLA-4 treatment and long term memory was significantly improved with the combination as shown in adoptive transfer studies. Notably, adoptive transfer of immune cells from animals that cleared their primary tumor through Toca 511, 5-FC, and αCTLA-4 showed 100% survival benefit to animals bearing orthotopic gliomas, significantly greater than the 50% survival seen with transfer from animals that cleared primary tumor through Toca 511 and 5-FC alone. Further, αCTLA-4 treatment during clearance of primary tumors resulted in a marked reduction of memory T regulatory cells in secondary tumors after adoptive transfer. To determine if αCTLA-4 in combination with Toca 511 and 5-FC could reduce primary tumor burden we developed a model using a sublethal infection level of Toca 511. Specifically, restricting Toca 511 infection to only 2% of tumor cells limited the tumor growth arrest activity of 5-FC and the loss of efficacy with 2% infection was worse when 5-FC treatment was combined with αCTLA-4. These data suggest that αCTLA-4, and other compounds that target Regulatory T cells, should be evaluated in patients receiving Toca 511 and 5-FC to determine if the combination confers additional clinical benefit.

Introduction

Toca 511 shows selectivity for tumor leading to conversion of the pro-drug, 5-FC, into 5-FU in the tumor microenvironment

![Diagram showing Toca 511 and 5-FC interaction with tumor cells]

Figure 1: Toca 511 is a retroviral replicating vector with the ability to spread through tumors (see top graph). When combined with 5-FU, high intratumoral concentrations of 5-FU result in significant reductions in tumor burden in a subcutaneous Tu-2449 glioma model. Toca 511 & 5-FC resulted in clearance of these tumors and therefore, further reductions in tumor burden by αCTLA-4 were not observed (A). As reported previously, Toca 511 and 5-FC depletes Myeloid Derived Suppressor cells (MDSC) and induces an increase in T cell infiltrate in the tumor microenvironment. Neither change was further augmented by the addition of αCTLA-4 (B and C). When a monoclonal antibody against CTLA-4 was combined with Toca 511 & 5-FC, Regulatory T cells were depleted from the tumor microenvironment (D).

Toca 511 and 5-FU induce anti-tumor immune memory which is further enhanced by combined treatment with αCTLA-4

![Graph showing time vs percentage survival with and without αCTLA-4]

Figure 2: Even though no further improvement in tumor burden was observed when Toca 511 & 5-FC was combined with αCTLA-4 monoclonal antibody therapy, regulatory T cells were depleted from the tumor microenvironment which suggested that effector T cell priming may be of a higher quality in animals that cleared their tumors through treatment with Toca 511 & 5-FC in the presence of αCTLA-4 compared to animals that cleared their tumors with Toca 511 and 5-FC alone. Splenocytes from donor mice that previously cleared Tu-2449 subcutaneous tumors with the above identified treatment modalities or naïve mice were adoptively transferred into mice with orthotopic Tu-2449 intracranial implants. This work showed that the addition of monoclonal antibody therapy against CTLA-4 did, in fact, result in improved anti-tumor immunity

Approach and Results

Toca 511 spreads within tumors. Initial infection of just 2% of tumor cells will result in high numbers of tumor cells becoming infected with time

![Diagram showing Toca 511 infected cell line and Toca 511 spreads through Tumor cells]

Figure 3: In order to limit the efficacy of Toca 511 & 5-FC and Toca 511, transduction superinfection resistance mechanisms were utilized to limit Toca 511 infection to only 2% of tumor cells (see graphic above). As few as 30% Toca 511 infected cells was sufficient to completely clear subcutaneous Tu-2449 tumors, treatment with 5-FU had similar efficacy to αCTLA-4 alone which was not significantly improved compared to control. Interestingly, the addition of αCTLA-4 significantly improved this response (B). To confirm, superinfection resistance was used to restrict Toca 511 infection to 2% of Tu-2449 cells that were implanted intracranially. The addition of αCTLA-4 significantly improved survival of animals receiving 5-FU as did the addition of PC-61 (αCD25 mAb) (C). PC-61 depletes regulatory T cells and this data suggests that compounds that deplete or limit Treg function may provide substantial benefits to overall efficacy of Toca 511 and 5-FC.

Conclusion

Combining Toca 511 & 5-FC with αCTLA-4 further enhances anti-tumor immune memory through depletion of Regulatory T cells

![Graph showing percent survival with and without combination therapy]

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