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A Retroviral Replicating Vector Encoding Cytosine Deaminase and 5-FC Induces Immune Memory in Metastatic Colorectal Cancer Models

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Treatment of tumors with Toca 511, a gamma retroviral replicating vector encoding cytosine deaminase, followed by 5-fluorocytosine (5-FC) kills tumors by local production of 5-fluorouracil (5-FU). In brain tumor models, this treatment induces systemic anti-tumor immune responses and long-term immune-mediated survival. Phase 1 Toca 511 and Toca FC (extended-release 5-FC) clinical trials in patients with recurrent high-grade glioma show durable complete responses and promising survival data compared to historic controls. The work described herein served to expand on our earlier findings in two models of metastatic colorectal carcinoma (mCRC). Intravenous (i.v.) delivery of Toca 511 resulted in substantial tumor-selective uptake of vector into metastatic lesions. Subsequent treatment with 5-FC resulted in tumor shrinkage, improved survival, and immune memory against future rechallenge with the same CT26 CRC cell line. Similar results were seen in a brain metastasis model of mCRC. Of note, 5-FC treatment resulted in a significant decrease in myeloid-derived suppressor cells (MDSCs) in mCRC tumors in both the liver and brain. These results support the development of Toca 511 and Toca FC as a novel immunotherapeutic approach for patients with mCRC. A phase 1 study of i.v. Toca 511 and Toca FC in solid tumors, including mCRC, is currently underway (NCT02576665).

INTRODUCTION

Toca 511 (vocimagene amiretrorepvec), a retroviral replicating vector, selectively replicates and spreads in malignant cells and encodes an optimized yeast cytosine deaminase (CD) protein. Toca 511 is designed to selectively infect cancer cells because retroviral replicating vectors (RRVs) selectively infect cancer cells because viral replication is restricted by innate and adaptive immune responses that are defective in malignant cells but intact in normal tissues.[1,2] As a further restriction to cancer cells, RRVs only infect actively dividing cells. In infected cells, CD enzyme is expressed and converts 5-fluorocytosine (5-FC) (an oral anti-fungal drug) to 5-fluorouracil (5-FU) (an anti-cancer drug). Our previous data demonstrated that Toca 511 administered intratumorally results in tumor cell death and provides a long-term survival benefit against brain cancer in preclinical models.[1,3–5] Administration of Toca 511 and subsequent treatment with the prodrug 5-FC is designed to generate higher levels of 5-FU in the tumor than can be achieved with systemic 5-FU delivery, allowing 5-FU tumor killing with fewer systemic toxicities. Direct tumor cytotoxicity and extended survival attributed to immunotherapeutic effects have been reported using this approach.[1,4–6]

Approximately 50% of patients with colorectal cancer (CRC) develop metastases (mCRC) during the course of the disease, with liver being the most frequent site. Standard treatment for mCRC is 5-FU-based combination therapy, which extends median survival from 6 to 24 months.[7] Brain metastases (BMs) from colorectal cancer are historically quite rare, representing only 4%–6% of all BM cases.[8,9] However, because first-line treatments are improving survival, brain metastasis is now becoming more frequent.[10,11] The current treatment options for BM from colorectal cancer include surgery or stereotactic radiosurgery, with or without whole-brain radiotherapy and, in rare cases, chemotherapy.[12] However, the prognosis for patients with BMs from colorectal cancer remains poor, with median survival ranging from 2 to 15 months.[13] Further, trials with new immunotherapeutic agents have had a limited therapeutic impact on mCRC.[14] Taken together, mCRC represents an area in which novel therapeutic approaches are desperately needed.

Recent studies suggest myeloid-derived suppressor cells (MDSCs) contribute to cancer immune evasion by suppressing anti-tumor immune response.[15–18] MDSCs are immature myeloid cells that are attracted to, and develop in, the tumor microenvironment by tumor-associated signals, and once there, continue to proliferate and actively suppress the immune system through multiple mechanisms. MDSCs are thought to be a key player in setting up and maintaining local immune suppression in numerous cancers, including mCRC.[19] There are no currently approved therapies specifically targeting

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MDSCs; however, it has been shown that 5-FU can deplete MDSCs,[20] presumably because of their continued proliferation and low levels of thymidylate synthase.[21] Therefore, treatment with Toca 511 and 5-FC to generate high local concentrations of 5-FU at the site of the tumor may have additional benefits outside of its ability to directly kill tumor cells. 5-FU may also confer an immunotherapeutic effect through depletion of highly immunosuppressive cells from the tumor microenvironment. There is a clear unmet medical need for new treatments for liver and brain metastases, and immunotherapeutic strategies that impact immunosuppressive tumor microenvironments appear to be useful candidates for this role. We show here that treatment with Toca 511 and 5-FC incorporates such a strategy.

We assessed the effects of Toca 511 and 5-FC treatment on survival and anti-tumor immune activation in syngeneic models of CRC metastases to the liver and brain. The results reported here support the development of Toca 511 and Toca FC as a novel immunotherapeutic approach for patients with mCRC and potentially other metastatic solid tumors. Toca 511 administered locally or intravenously (i.v.) combined with oral Toca FC (extended release 5-FC) is under investigation in patients with recurrent primary brain tumors (NCT01156584, NCT01470794, NCT02414165, and NCT01985256). Potential benefits have been observed, including durable complete responses, extended overall survival compared to historic controls, and a favorable safety profile.[22] A phase 1 study of i.v. Toca 511 followed by cycles of oral Toca FC in patients with solid tumors, including mCRC, is currently underway (NCT02576665).

RESULTS

Toca 511 in Combination with 5-FC Prolonged Survival in a Liver Metastasis Model of mCRC

Survival was assessed in a multifocal liver metastasis model of murine mCRC after treatment with Toca 511 and 5-FC. In order to monitor tumor take and progression, CT26 cells were engineered to express luciferase (CT26-Luc) and mice were monitored through noninvasive imaging throughout the study (Figures 1A, S1A, and S1B). Mice that were inoculated intrasplenically with Toca 511 pre-transduced CT26-Luc cells developed liver metastases as early as 6 days after inoculation. 5-FC treatment cycles were initiated on day 13 and continued for a total of 6 cycles. All animals in the PBS control group showed tumor progression, as evidenced by increased bioluminescence signal over time. The Toca-511- and 5-FC-treated animals showed slower tumor progression than the control group after 6 cycles of 5-FC treatment. On average, progression in the Toca 511 and 5-FC treatment group was blunted over time and stabilized through cycles of 5-FC (Figures 1B and S1B). Treatment with 5-FC resulted in prolonged survival compared to PBS control ($p = 0.05$) (Figure 1C). Five of 9 tumor-bearing mice (55%) remained tumor free after cessation of 5-FC until the end of the study (day 90). Prolonged survival after cessation of treatment suggested that survival was at least partially due to the induction of anti-tumor immune response. To further evaluate the effect of Toca 511 and 5-FC treatment on the induction of anti-tumor immune responses, CT26 cells were implanted into the right flanks of "cured" mice as well as naive, age-matched mice on day 90 after the original intrasplenic tumor implant. Tumors engrafted and grew in all naive animals; however, tumors were rejected in mice that had previously cleared CT26 liver metastases through treatment with Toca 511 and 5-FC ($p = 0.028$ versus naive) (Figure 1D). Animals that received Toca 511 and 5-FC did not exhibit signs of toxicity (Table S1).

Toca 511 Efficiently Infects and Spreads in Multifocal Liver Metastases after Intrasplenic, i.v., or Intraportal Delivery

In order to obtain maximal spread of Toca 511, initial distribution of vector through an optimal delivery modality is a key factor. In this study, instrasplenic, i.v., and intraportal vein routes were compared to evaluate vector delivery to multifocal liver metastases. 3 days post cell inoculations, one dose of vector (3.4×10^7 TU) was delivered via three different routes. A GPF-expressing vector (Toca GFP) was utilized in order to facilitate imaging of the vector within metastases in the liver. Metastatic lesions, as mentioned above, expressed luciferase and were therefore visualized by bioluminescence imaging. 18 days post vector administration, all delivery routes resulted in vector expression in tumor foci, but not in normal liver tissue (Figure 2A). Excised multifocal liver metastases were also analyzed by flow cytometry. The average percentage of GFP^+ cells for intrasplenic, i.v., and intraportal delivery modalities were similar: 7.8 ± 0.9, 8.2 ± 1.2, and 6.2 ± 1.6, respectively (Figure 2B). i.v. vector administration was selected for all subsequent experiments. A total of three consecutive doses of Toca GFP versus a single dose of i.v. delivery of Toca GFP resulted in higher average GFP^+ cells, as seen by vector spread at 14 and 22 days post vector administration (Figure 2C).

i.v. Delivery of Toca 511 and Treatment with 5-FC Was Efficacious in a Murine Liver Metastasis Model of mCRC

Although pre-transduced CT26 cells provided insight into the feasibility of treating metastatic disease with this therapeutic platform, we wanted to confirm these results in a clinically relevant delivery model. Therefore, in order to assess the therapeutic efficacy of 5-FC treatment after i.v. delivery of Toca 511, an additional survival study was conducted. Mice were administered 5 consecutive (one injection per day for 5 days) i.v. injections of Toca 511 (6.6×10^7 TU/injection/day) starting 4 days post tumor cell inoculation, followed by six cycles of 5-FC. Animals that were treated with 5-FC lived significantly longer compared to PBS control ($p = 0.037$) (Figures 3A, S1A, and S2B). Six of 9 mice remained tumor free, even after cessation of 5-FC (Figures 3B and S2B). Complete blood counts were collected at 17, 25, and 35 days post cell inoculations. 5-FC treatment caused a transient decrease in white blood cell (WBC), lymphocyte (LYM), neutrophil (NEU), and platelet (PLT) counts after the first and second 5-FC cycles compared to PBS treatment. However, values were above the lower limits of normal (LLN) by the end of the fourth cycle of 5-FC treatment (Figure 3D). Modest lymphoid suppression was observed with the use of Toca 511 and 5-FC relative to systemic 5-FU. As above, CT26 cells were implanted into the right flanks of cured as well as naive, age-matched, mice on day 90 after the original intrasplenic tumor implantation. Tumors engrafted and grew in all naive animals; however, tumors were rejected in mice that had previously cleared CT26 liver metastases through treatment with Toca 511 and 5-FC ($p = 0.001$) (Figure 3C).