Background

For decades, radiotherapy (RT) has been a cornerstone of cancer treatment. Currently, approximately 50% of cancer patients will be treated with RT. Beyond the ability of RT to produce free radicals and to generate single and double-strand breaks in DNA, triggering cell death, preclinical and clinical studies have demonstrated that RT can have immunomodulatory effects. For example, RT can stimulate MHC class I expression on cancer cells, induce immunogenic cell death (ICD), and activate expression of various pro- and anti-inflammatory cytokines and adhesion molecules, allowing recruitment and activation of both innate and adaptive immune cells into the tumor. Unfortunately, RT rarely produces a sustained antitumor response as immune escape frequently occurs with tumor recurrence. Moreover, the so-called ‘abscopal effect’ which corresponds to reduction of metastatic burden outside the irradiated area is rarely observed after RT. Finally, the maximum dose of irradiation is limited because of toxicity to surrounding healthy tissues.

The high electron density of functionalized hafnium oxide nanoparticles (NBTXR3) allows a high probability of interaction with incoming ionizing radiation, increasing energy dose deposit within cells. We have previously reported in nonclinical studies the ability of RT-activated NBTXR3 (NBTXR3+RT) to increase cancer cell destruction as well as better control of treated tumor growth through this physical mode of action leading, compared to RT alone. Furthermore, NBTXR3+RT demonstrated clinically meaningful benefit for patients with locally advanced Soft Tissue Sarcoma compared to RT alone, in the randomized controlled phase II/III Act.in.Sarc study (NCT02379845).

Methods

To explore the impact of NBTXR3+RT on the anti-tumor immune response, we used CT26 mouse colorectal cancer cells to perform a series of abscopal assays in immunocompetent mice.

Results

We showed that NBTXR3+RT can generate a significant abscopal effect along with a substantial increase of CD8+ T cell infiltration both in treated and untreated tumors, compared to RT alone. We showed that this distant effect was fully dependent on CD8+ T cells, as their depletion completely abolished the abscopal effect. To better understand how NBTXR3+RT treatment could generate this abscopal effect, we compared the TCR repertoire of treated and untreated tumors for the different conditions. This analysis revealed that NBTXR3+RT was able to broaden clonal diversity in both treated and untreated tumors, compared to RT alone.

Conclusions

This indicates that NBTXR3+RT has the ability to transform the tumor into a ‘in situ vaccine’ more efficiently than RT alone and could have important implications for the use of NBTXR3+RT in combination with immunotherapy.

Ethics Approval

All animal experiments were carried out in compliance with French and European laws and regulations (European Directive 2010/63 EU). The local institutional animal ethics board and French Ministère de la Recherche approved all mouse experiments (permission numbers: 2016_031_4340 and 2016_129_8344).

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and colon cancer lines, CRISPR/Cas9-mediated gene editing was used to differentiate the transcriptomic profile driven by HIF-2α from that of HIF-1α or HIF-3α, allowing for the derivation of a HIF-2α-specific gene signature. Cancer cell and macrophage-derived signatures were applied to publicly available datasets to investigate cancer types, other than ccRCC, in which HIF-2α may play an important pathological role.

Conclusions Collectively, these data support the development of our novel and selective HIF-2α inhibitor, which may play an important pathological role.

Methods

To test this hypothesis, melanoma-bearing mice were treated intratumorally with VN agents. Five days post-treatment, tumors were digested into single cell suspensions and RNA was isolated and used for RT-PCR. Transcript levels of TLS-promoting factors (CCL19, CCL21, CXCL13) and markers of vascular normalization (HIF1α, GLUT1) and inflammation/immune cell infiltration (CXCL10, VCAM1, CD8A) were measured and compared to PBS treated controls. Changes in tumor vasculature were evaluated using immunofluorescence microscopy (IFM) of tumor sections stained with CD31, PNAd, and PDGFRβ. Fluorescently-labeled lectin was injected into the mice to observe tumor perfusion. TLS formation was evaluated in tumor sections using IFM, with TLS being defined as PNAd+ vessels surrounded by clusters of CD45+ cells.

Results We observed that the VN agents dasatinib, STING agonist, bevacizumab, and agonist anti-TNFβ1 antibody each induced global changes in the TME that are consistent with enhanced immune cell infiltration and TLS formation. These changes include increases in expression of CCL19, CCL21, and VCAM1. Dasatinib and STING agonists were shown to promote VN, as demonstrated by improved lectin perfusion into the tumor and increased pericyte coverage of blood vessels on-treatment.

Conclusions VN agents induce global changes in immune cell infiltration and TLS-promoting factors in the TME. In vivo, these agents induce VN in the TME and promote TLS formation. This knowledge can be used to develop optimal combination immunotherapy designs in the cancer setting.

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585 INTRALESIONAL INJECTION OF ROSE BENGAL IMPROVES THE EFFICACY OF GEMCITABINE CHEMOTHERAPY AGAINST PANCREATIC CANCER

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Background Chemotherapy regimens that include gemcitabine are considered standard of care in patients with advanced pancreatic ductal adenocarcinoma (PDAC). However, most patients with PDAC die within 2 years of diagnosis, even with these standard of care regimens. In this study, we explored the ability of intratumoral injections of PV-10, a 10% solution of rose bengal, to induce lesion-specific ablation and control of metastatic pancreatic tumors in a murine model.

Methods PV-10 was cultured with human pancreatic cancer cell lines overnight and cell death was measured via Annexin-V and DAPI staining. Murine pancreatic tumor cells (Panc02) were injected subcutaneously in one flank to establish a single tumor model; to establish a bilateral tumor model, Panc02 tumor cells were implanted in the opposite flanks. On day 7, a single tumor was treated with intralesional PV-10. Gemcitabine (60 mg/kg) was injected intraperitoneally twice per week for 2 weeks. These experiments were repeated using Panc02 cells modified to overexpress the neoantigen ovalbumin (OVA). Control mouse tumor were directly injected with PBS. Tumor growth of PV-10 injected tumors and non-injected bystander tumors on the opposite flank were measured. Damage associated molecular patterns (DAMPs) in serum and immune cell frequencies within the spleens of tumor-bearing mice were measured to identify an associated systemic response with tumor lytic treatment regimen.

Results We established that less than 50% of human and murine pancreatic cells were alive after a 24 hour incubation with 200µM PV-10 in vitro. The combination of intralesional PV-10 with the systemic administration of gemcitabine delayed the growth treated tumors and non-injected distal tumors. In contrast, gemcitabine monotherapy failed to delay tumor growth in bilateral Panc02 tumor models. We observed that this treatment strategy was markedly more successful in immunogenic Panc02OVA tumors resulting in lesion-specific ablation in 5/8 mice compared to 0/8 mice that were treated with gemcitabine monotherapy. This suggests that the combination therapy enhanced the immune-mediated clearance of tumors. Moreover, regression of tumors in mice that received PV-10 in combination with gemcitabine was associated with the depletion of splenic CD11b+Gr-1+ cells and increases in damage associated molecular patterns HMGB1, S100A8, and IL-1α.

Conclusions Together, these results demonstrate that intrallesional therapy with PV-10 can enhance the efficacy of gemcitabine against pancreatic tumors.

Ethics Approval Studies were performed under approved Institutional Review Board (IRB) laboratory protocols at the H. Lee Moffitt Cancer Center (Tampa, FL).

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