

the tumors. None of the combination therapy mice had grossly detectable lung metastases at time of death but metastases were present in the trabectedin only (20%), LOFU only (50%), and control (50%) groups (not statistically significant). **Conclusions** Combination therapy with trabectedin and LOFU yielded smaller tumor size and fewer pulmonary metastases compared to individual therapies alone.

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## 582 MODULATION OF TCR REPERTOIRE BY RADIOTHERAPY-ACTIVATED NBTXR3 NANOPARTICLES

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**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 anti-tumor 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 infiltrates 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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## 583 NOVEL, POTENT, AND SELECTIVE INHIBITORS OF HYPOXIA-INDUCIBLE FACTOR (HIF)-2 $\alpha$ REVERSE PRO-TUMORIGENIC TRANSCRIPTIONAL PROGRAMMING IN CANCER, STROMAL, AND IMMUNE CELLS

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**Background** The microenvironment of solid tumors is hypoxic and requires induction of genes associated with metabolism, growth, proliferation, and angiogenesis for cancer cells to survive and metastasize. The master transcriptional regulators of hypoxia-induced genes are the HIF proteins, consisting of three distinct oxygen-regulated  $\alpha$  monomers (HIF-1 $\alpha$ , -2 $\alpha$ , and -3 $\alpha$ ). In normoxia, hydroxylation of HIF-2 $\alpha$  allows for recognition by the pVHL E3-ubiquitin ligase complex and proteasomal degradation. Exposure to hypoxia, or VHL mutation or silencing, leads to HIF-2 $\alpha$  stabilization, dimerization with HIF-1 $\beta$ /ARNT, and transcription of pro-tumorigenic gene sets in a variety of cancer and non-cancer cell types in the tumor microenvironment. In patients, overexpression of HIF is associated with poor prognosis, and both preclinical and clinical evidence suggests that inhibiting HIF-2 $\alpha$  is an effective strategy to mitigate tumor growth, particularly in clear cell renal cell carcinoma (ccRCC), warranting further development of HIF-2 $\alpha$  inhibitors and investigation into the role of HIF-2 $\alpha$  in various cellular and combinatorial settings.

**Methods** Using a suite of assays to evaluate HIF-2 $\alpha$ -specific effects, herein we describe pharmacological properties associated with novel, potent, and selective small-molecule inhibitors of HIF-2 $\alpha$ .

**Results** Optimized compounds inhibited HIF reporter transcription and VEGF secretion. Compounds that were biochemically confirmed to bind HIF-2 $\alpha$  also inhibited HIF-2 $\alpha$ , but not HIF-1 $\alpha$ , mediated gene expression. Characterization of HIF-2 $\alpha$  inhibition was expanded to human stromal and immune cell subsets. While compounds inhibited pro-angiogenic gene sets in endothelial cells, inhibiting HIF-2 $\alpha$  in activated hypoxic T cells did not affect proliferation or cytokine secretion, suggesting that HIF-2 $\alpha$  inhibitors would not impede T cell functionality in tumors. In contrast, in a M2-polarized macrophage model for suppressive tumor-associated macrophages, HIF-2 $\alpha$  drove hypoxia-induced changes in the chemokine secretome that favored granulocytic rather than lymphocytic infiltration, an effect that was effectively reversed by HIF-2 $\alpha$  inhibition. At the transcriptional level, mRNA-sequencing was used to define global gene sets impacted by HIF-2 $\alpha$  inhibition in M2 macrophages. Additionally, in a set of liver, kidney, pancreatic,