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**RADIOTHERAPY-ACTIVATED NBTXR3 NANOPARTICLES INCREASE CD8+ T CELL INFILTRATION AND DIVERSITY IN TUMORS, AND MODULATE THE IMMUNOPEPTIDOME OF CANCER CELLS**Audrey Darmon, Ping Zhang, Jordan Da silva\*, Sebastien Paris. *Nanobiotix, Paris, France*

**Background** When exposed to radiotherapy (RT), NBTXR3 nanoparticles increase radiation dose deposition from within the cancer cells. NBTXR3 is intended for a single intratumor injection. Results from a phase II/III clinical trial in patients with locally advanced Soft Tissue Sarcoma demonstrated significant superiority and clinical benefits of NBTXR3 activated by RT compared to RT alone, and was well tolerated. NBTXR3 is currently being evaluated in several other tumors including head and neck, liver, and pancreatic cancer as a single agent or in combination with anti-PD1. Moreover, preclinical studies have demonstrated that NBTXR3 can produce a significant abscopal effect, whereas RT alone cannot. Here, we explored the impact of NBTXR3 activated by RT on CD8+ infiltrates and TcR repertoire diversity change, and the effect on the immunopeptidome of cancer cells.

**Methods** CT26 (murine colorectal cancer cells) were subcutaneously injected in BALB/c mice in one flank. Then, tumors were intratumorally injected with NBTXR3 (or vehicle) and irradiated 24 hours later with 4Gy per fraction for 3 consecutive days. Tumors were collected 3 days after the last RT fraction and immune cell infiltrates were measured using immunohistochemistry (IHC) and digital pathology. For TcR repertoire sequencing, the same workflow was followed, except cells were injected in both flanks. Only right tumors received treatment, while left tumors remained untreated. For immunopeptidome analysis, in vitro cells were irradiated by 4Gy. After one day, cells were collected for isolation and sequencing of MHC I-loaded peptides.

**Results** IHC analyses showed a significant increase of CD8+ T cell infiltrates in tumors of mice treated with NBTXR3+RT, while RT alone had no significant effect. In addition, NBTXR3+RT treatment was able to increase TcR repertoire diversity, both in treated and untreated tumors, compared to RT alone. Finally, analysis of immunopeptidome showed that NBTXR3+RT changed the profile of MHC-I-loaded peptides.

**Conclusions** Our in vivo data indicate that NBTXR3+RT can modulate the microenvironment of treated tumors, leading to enhanced CD8+ T cell infiltration as well as modification of the TcR repertoire, both in treated and distant untreated tumors. These NBTXR3+RT-induced responses may be related to changes in the immunopeptidome of cancer cells triggered by this treatment.

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