Title
Effective antitumor immunity with hafnium oxide at the nanoscale

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Background
Radiation therapy (RT) has demonstrated ability to augment antitumor immunity, promoting immunogenic cell death (ICD) and stimulating immune adjuvant effects. On the other hand, RT has also been reported to induce immunosuppressive responses.
A new class of material with high electron density, hafnium oxide, was designed at the nanoscale (HfO_2-NP) to efficiently absorb ionizing radiation and augment the radiation dose deposited from within the tumor cells. HfO_2-NP, administered via a single intratumor injection, is currently evaluated in clinical trials including soft tissue sarcoma, head and neck, prostate, liver and rectum cancers.
Here, we explore the ability of HfO_2-NP exposed to RT to bring a substantial effect on antitumor immunity.

Methods
The potential ability of HfO_2-NP exposed to RT to transform tumors into immunologically active lesions was tested *in vitro* and *in vivo*.
In vitro, the level of ICD markers was evaluated in a panel of human cancer cell lines, following cells treated or not with HfO_2-NP and irradiated.
In vivo, a vaccination assay was performed to evaluate the host immune responses in immunocompetent mice inoculated with murine CT26 cancer cells treated or not with HfO_2-NP and irradiated with 6 Gy. In addition, one study evaluated the level of immune cells infiltration in tumors using the same model: mice bearing CT26 tumors in the left and right flanks were treated (right tumor only) or not with HfO_2-NP and exposed to 4Gy for 3 consecutive days.

Results
Higher DAMPs levels (cell surface expression of calreticulin, extracellular adenosine triphosphate level and extracellular high-mobility group box 1 level) were observed in the tested cancer cells treated with HfO_2-NP + RT when compared to cancer cells exposed to RT.
In the vaccination assay, 40 days after the challenge phase (injection with untreated CT26 cells), the percentage of tumor-free mice was markedly higher for mice vaccinated with cells treated *in vitro* with HfO_2-NP + RT compared to those vaccinated with cells treated *in vitro* with RT. In another study applying the principle of abscopal assay, a marked increase of $CD8^+$ T cell lymphocytes and macrophages (CD68) infiltrates was observed in both tumors (treated and untreated tumors), 72 hours after the last RT session, for mice treated (right flank) with HfO_2-NP + RT. On the contrary, no effect was observed in both tumors for mice treated (right flank) with RT.

Conclusion
These results suggest an efficient cell killing (ability to generate ICD) with superior potential of HfO_2-NP + RT to transform the tumor into an effective *in situ* vaccine when compared to RT. Moreover, HfO_2-NP treatment generates a marked increase of immune cells infiltration in the tumors suggesting that it may convert immunologically “cold” tumor into “hot” tumor and could be combined with immunotherapeutic agents across oncology.