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**ABSTRACT TITLE** Identification of a class of non-conventional ER-stress-response-derived immunogenic peptides

**Background** Efforts to overcome resistance to immune checkpoint blockade therapy have focused on vaccination strategies using neoepitopes, although they cannot be applied on a large scale due to the ‘‘private’’ nature of cancer mutations. We have previously shown that infection of melanoma cells with Salmonella induces the transfer of antigens between adjacent cells through gap junctions, formed by the docking of two hemichannels. We hypothesized that Salmonella may also lead to a hemichannels-mediated extracellular release of immunogenic peptides (secretome) by tumor cells that could be used as anticancer vaccine.

**Methods** Salmonella enterica serovar Typhi Ty21a was used to infect tumor cells. Immunogenicity of secretome was tested on mouse model of melanoma and in a therapeutic clinical trial for osteosarcoma dog patients. Peptide sequences were identified by peptidomic analysis on secretome of Salmonella-infected tumor cells. Knock-out tumor cells to assess the involvement of ER-genes in the mechanism of peptide-release were generated by CRISPR-Cas9.

**Results** We show that infection of tumor cells with Salmonella induces the opening of membrane hemichannels and the extracellular release of proteasome-generated peptides by the exacerbation of endoplasmic reticulum (ER) stress. Peptides released by cancer cells foster an antitumor response in vivo, both in mice bearing B16F10 melanomas and in dogs suffering from osteosarcoma. Mass spectrometry analysis on the supernatant of human melanoma cells revealed 12 peptides capable of priming healthy-donor $CD8^+$ T cells that recognize and kill human melanoma cells in vitro and when xenotransplanted in vivo, but not melanocytes.

**Conclusions** We identified a class of shared tumor antigens that are generated in ER-stressed cells, such as tumor cells, that do not induce tolerance and are not presented by healthy cells. An ERstreP-based ‘universal’ vaccine would lead to an ‘off the shelf’ vaccination treatment that could be administered to patients soon after tumor resection, rapidly boosting an antitumor response directed against micro-metastases that are often not clinically visible at the moment of primary tumor diagnosis. To enhance the efficacy of the cancer vaccine it will be pivotal to consider a combination with ICB to improve patients clinical benefit.

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