Background Intravesical administration of the tuberculosis vaccine Bacillus Calmette-Guérin (BCG) was the first immunotherapy to be approved by the FDA and is still nowadays the treatment of choice for a subset of non-muscle invasive bladder cancer patients. Intravenous (IV) administration of BCG has been recently reported to be highly effective at preventing tuberculosis infection in macaques by inducing strong T cell responses in the lung, as well as to alter myelopoiesis, generating “trained” macrophages which confer protection against tuberculosis infection in the lung. Therefore, in this work we set out to characterize the therapeutic application of IV BCG in mouse models of lung cancer.

Methods We used mouse models of tumors growing in the lung based on IV inoculation of B16-F10 melanoma and LLC lung carcinoma tumor cells to study the therapeutic effect of IV BCG. We performed immune cell profiling by flow cytometry to study the mechanism of action, as well as functional assays such as NK and T cell cytotoxicity assays.

Results First, we observed that therapeutic IV BCG administration extended mice survival in models of B16-F10 lung metastasis and orthotopic LLC lung tumors (figure 1). Immune cell depletion studies revealed that NK cells and CD4+ and CD8+ T cells were required for IV BCG efficacy against lung tumors (figure 2). IV BCG induced a tumor-specific CD8+ T cell response in the lung in a process relying on type 1 conventional dendritic cells (cDC1s). Mechanistically, BCG stimulated lung NK cells (figure 3A), which participated in the recruitment of cDC1s to the tumor (figure 3B) and initiated tumor-specific T cell responses (figure 3C) by favoring the loading of cDC1s with tumor-associated material by killing tumor cells in a perforin-dependent manner (figure 4). Remarkably, IV BCG reduced tumor growth in B16-F10 and LLC lung tumors in which MHC-I expression was ablated (figure 5). Finally, we observed that IV BCG upregulated PD-L1 in multiple immune cells in the tumor microenvironment (TME), suggesting the generation of an inflamed TME. Combination of IV BCG and antiPD-L1 antibodies further increased mouse survival in mouse models of lung tumors, which were otherwise resistant to checkpoint blockade as a monotherapy (figure 6).

Conclusions Here we describe a therapy based on IV BCG administration which enabled antitumor immunity in the lung by coordinating multiple immune cell populations, including both T and NK cells as well as Batf3-dependent cDC1s. Remarkably, IV BCG sensitized lung tumors to antiPD-L1 checkpoint blockade therapy.

REFERENCES

Ethics Approval Experimental work was conducted in agreement with European and national directives for protection of experimental animals and experimental procedures were approved by the Ethics Committee for Animal Experiments of University of Zaragoza (PI46/18, PI33/15 PI50/14).