receptors, P2RX7 is preferentially expressed in immune cells. Notably, we recently discovered that P2RX7 is crucial for the generation and maintenance of long-lived tissue-resident and circulating memory CD8+ T cells.1 2 CD8+ T cell function is fundamental for tumor control, and therapies to harness protective CD8+ T cells that overcome exhaustion are currently in the limelight of anticancer strategies. Given our previous data, and the fact that eATP is abundantly present inside the melanoma microenvironment, we tested whether (a) P2RX7 is required for activated CD8+ T cells to infiltrate and control melanoma upon adoptive cell therapy, and (b) P2RX7 agonism can boost the anticancer capacity of CD8+ T cells.

Methods (a) We in vitro-activated WT or P2rx7-/- CD8+ T cells (transgenic for the LCMV epitope gp33-P14 or for the ovalbumin SIINFKEK peptide-OTI) with anti-CD3/CD28/IL-2, ± Bz-ATP, a P2RX7 agonist, mitochondrial respiration via Seahorse) were measured in immune cell phenotyping. Some parameters (cytokine production, mitochondrial respiration via Seahorse) were measured in in vitro-activated cells. (b) WT and P2rx7-/- cells were activated with anti-CD3/anti-CD28/IL-2, ± Bz-ATP, a P2RX7 agonist. Tumor growth was tracked over time until 60 days or at the appropriate endpoint.

Results WT and P2RX7-deficient (P2rx7-/-) CD8+ T cells in the absence of IL-12 do not differ in tumor infiltration and/or control. However, P2rx7-/- CD8+ T cells activated in response to IL-12 tertiary stimulus do not control B16 melanomas as well as their WT counterparts. Phenotypically, IL-12-P2rx7-/- CD8+ T cells do not profoundly differ from IL-12-WT CD8+ T cells, except for diminished mitochondrial respiration levels in vitro, and diminished mitochondrial membrane potential (e.g. mitochondrial health) among tumor-infiltrating cells. Strikingly, Bz-ATP treatment increased the mitochondrial activity of WT CD8+ T cells in vitro and in vivo and led to increased B16 infiltration and control, in a P2RX7-dependent manner.

Conclusions We are currently studying the mechanisms behind the ability of P2RX7 agonists to increase the antitumor function of CD8+ T cells; these are promising results that can lead to a new alternative in immune cell therapies against melanoma.

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Ethics Approval This study was approved by the IACUC board at the University of Minnesota (IACUC number A3456-01).

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