Background Hypofractionated radiotherapy (RT) combined with anti-CTLA-4 antibodies has been shown to induce systemic anti-tumour immunity in anti-CTLA-4-refractory metastatic non-small cell lung cancer (NSCLC) patients. The immunogenicity of RT relies on its activation of conventional type I dendritic cells (cDC1s) which are essential for cross-presentation of tumour-specific CD8 T cells, a process which is regulated by CTLA-4. We recently showed that tumour expression of mono-ADP-ribosyltransferase-1 (ART1) mediates immune tolerance by using NAD⁺ to mono-ADP-ribosylate (MARylate) the P2X7 receptor (P2X7R) leading to NAD-induced cell death. Here, we assess whether P2X7R-expressing cDC1s are targeted by ART1, whether ART1-blockade modulates RT-mediated cDC1 tumour infiltration, and whether ART1-blockade potentiates synergy between RT and CTLA-4-blockade in the immunotherapy-resistant Lewis Lung Carcinoma (LLC1) model.

Methods An etheno-NAD (e-NAD) assay was used to assess MARylation of bone marrow-derived cDC1s co-cultured with recombinant ART1 (rART1) and ART1-blockade using a novel monoclonal ART1 blocking antibody, 22C12. C57BL/6j mice were inoculated with LLC1 flank tumours on day 0 and randomized into treatment groups: (1) iso ctrl, (2) aCTLA-4, (3) aART1, (4) aCTLA-4+aART1, (5) RT+iso ctrl, (6) RT+aART1, and (8) RT+aCTLA-4+aART1. Image-guided RT was delivered to the tumours on day 7–9 as 8 Gy fractions on consecutive days. ART1-blockade using 22C12 and CTLA-4-blockade using 9H10 started on day 7 and 9 respectively. Antibodies were delivered intraperitoneally every three days until the end of study. Tumours were harvested for flow cytometry analysis on day 14 (non-irradiated mice) and on day 23 (irradiated mice).

Results cDC1s were MARylated in the presence of rART1 primarily affecting the P2X7R+ cDC1 population that lacked co-expression of the NAD-cyclase CD38. In this population, 76.1 ± 4.8% of cells were MARylated following co-culture with rART1 compared with 16.9 ± 18.3% of cells cultured with e-NAD alone. Addition of ART1-blockade reduced MARylation of P2X7R+CD38- cDC1s to 26.1 ± 19.3% (figure 1A). ART1-blockade resulted in a significant tumour enrichment of cDC1s in irradiated mice (p<0.01) but not in non-irradiated mice (figure 1B). In irradiated mice, combined ART1-blockade and CTLA-4-blockade delayed tumour progression of LLC-1 tumours compared to RT alone (p<0.01), which was not observed with aCTLA-4 or aART1 alone (figure 1C).

Conclusions Our findings indicate that ART1 MARylates cDC1s in vitro and abrogates radiation-induced tumour infiltration of cDC1s in vivo. Further, ART1 blockade potentiates the anti-tumour effect of RT and CTLA-4-blockade in an immunotherapy-resistant lung cancer model, warranting further exploration of ART1-blockade in NSCLC to increase patient responsiveness to CTLA-4-blockade and radiotherapy.

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