Antibody Blockade of the Mono-ADP-Ribosyltransferase ART1 Promotes Radiation-Induced Tumour Infiltration of Dendritic Cells and Potentiates Tumour Control by Radiotherapy and CTLA-4 Inhibition

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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 resistance in non-small cell lung cancer. 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 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±19.8% (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 LLC1 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 vitro 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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Abstract 868 Figure 1 (A) Etheno-NAD (e-NAD) assay determining MARylation of bone marrow-derived cDC1s. cDC1s were incubated for 2 hours with e-NAD alone or with e-NAD and recombinant ART1 (rART1) in the presence or absence of 22C12 ART1-blocking antibody (aART1). After coculture, cDC1s were analysed by flow cytometry for ART1-mediated MARylation by e-NAD staining and co-stained for P2X7R and the NAD-cyclase CD38. One-way ANOVA with Tukey’s test for multiple comparisons. (B) Percentage of cDC1 cells among total tumour-infiltrating DCs in LLC1 tumours. Non-irradiated tumours (no RT) were harvested on day 15 and irradiated tumours (RT) were harvested on day 24 after tumour inoculation. Two-tailed t-test with Welch’s correction. (C) Growth curve of subcutaneous flank LLC1 tumours (n=5 mice/group). Two-way repeated-measures ANOVA. *p<0.05, **p<0.01, ****p<0.0001.