

The ART of tumor immune escape

Erik Wennerberg^{a,*}, Sumit Mukherjee^{b,*}, Ricardo M. Sainz^a, and Brendon M. Stiles^b

^aDivision of Radiotherapy and Imaging, Institute of Cancer Research, London, UK; ^bDepartment of Cardiothoracic and Vascular Surgery, Albert Einstein College of Medicine, Bronx, NY, USA

ABSTRACT

We recently identified the adenosine-5'-diphosphate (ADP)-ribosyltransferase-1 (ART1) as a novel immune checkpoint expressed by cancer cells. ART1 utilizes free nicotinamide adenine dinucleotide (NAD⁺) in the tumor microenvironment (TME) to mono-ADP-ribosylate (MARylate) the P2X7 receptor (P2X7R) on CD8 T cells, resulting in NAD-induced cell death (NICD) and tumor immune resistance. This process is blocked by therapeutic antibody targeting of ART1.

ARTICLE HISTORY

Received 21 April 2022
Revised 4 May 2022
Accepted 5 May 2022

KEYWORDS

mono-ADP-ribosylation;
immune escape; CD8 T cells;
NAD-induced cell death;
P2X7 receptor; CD38; lung
cancer

Adenosine-5'-diphosphate (ADP)-ribosylation is an evolutionarily conserved regulatory mechanism used by several bacterial toxins to modify host cell proteins involved in the pathogenesis of common human diseases. ARTD (diphtheria toxin-like) family mono-ADP-ribosyltransferases (mARTs), including poly-ADP-ribose-polymerase (PARP)7 and PARP16, are increasingly recognized as promising therapeutic targets for cancer.¹ In contrast to these intracellular mARTs, the less studied ARTC (clostridia toxin-like) family members, which include ART1, are glycosylphosphatidylinositol (GPI)-linked and can be expressed on the cell surface.² Although most gene expression databases show low *ART1* expression levels, ART1 protein is strongly expressed by many human cancers in the Human Protein Atlas. While ART1 may have intrinsic pro-tumor effects via MARylation of cancer cell surface receptors, previous studies describing how ART2 (an ARTC family member not expressed in humans) regulates tissue immune homeostasis in models of acute inflammation, raised the intriguing possibility that ARTC family members could modulate the immune landscape also in tumors. Adriouch, Stark et al. describe how bystander (antigen-naïve) T cells are eliminated by NAD-induced cell death (NICD) via ART2-mediated MARylation of P2X7 receptor (P2X7R) in cis, while antigen-primed T cells reduce their susceptibility to NICD by downregulating ART2 and P2X7R and by upregulating the ecto-NADase CD38 to remove free NAD⁺ from their microenvironment. Thus, ART-mediated NICD constitutes an immune homeostatic mechanism that favors persistence and expansion of antigen-specific T cells.^{3,4}

In our recently published manuscript, we provide the first evidence that tumors co-opt the NICD pathway by upregulating ART1 and MARylating P2X7R on tumor-infiltrating CD8 T cells in trans, leading to their elimination by NICD (Figure 1).⁵ CD38 expression, which is upregulated on T cells upon activation and

differentiation, appears cytoprotective as blockade of CD38 enhanced MARylation and NICD. We further demonstrate that knockdown or blockade of ART1 promoted infiltration of P2X7R⁺ CD8 T cells and reduced tumor burden in orthotopic models of lung cancer and melanoma. Similarly, in human lung tumors, we demonstrated that higher ART1 protein expression was associated with decreased infiltration of P2X7R⁺ CD8 T cells. Analysis of data from The Cancer Genome Atlas (TCGA) showed that high *ART1* mRNA expression was associated with low expression of CD8 T cell cytotoxicity and immunoregulatory genes.

Notably, as an extracellular enzymatic target, ART1 should be highly druggable. To that end, we developed therapeutic monoclonal antibody candidates for preclinical studies. Our lead candidate, 22C12, effectively bound to both mouse and human ART1 and potently inhibited ART1 enzymatic activity. Intratumoral and systemic deliveries of 22C12 increased tumor infiltrating P2X7R⁺ CD8 T cells and decreased tumor burden in flank and orthotopic tumor models. Treatment with 22C12 increased the proportion of Ki67⁺ and programmed cell death-1 (PD-1)⁺ CD8 T cells. When we further evaluated distinct populations of P2X7R⁺ CD8 T cells, we found a notable increase in the absolute number of tissue resident memory (T_{RM}: CD62L⁻CD44⁺CD69⁺) P2X7R⁺ CD8 T cells. This raises interesting questions regarding the role of P2X7R expression and ART1-induced NICD in CD8 T cell development and in regulation of other populations of immune cells in the TME. P2X7R is known to be widely expressed in immune cells, where activation by extracellular ATP is generally thought to stimulate antitumor immunity.⁶ Correspondingly, in some murine models, tumor development has been shown to be more aggressive in P2X7R-deficient animals.⁷ In line with this observation, recent work has demonstrated that P2X7R expression is critical for memory CD8 T cell differentiation and that P2X7R^{high} CD8 T cells

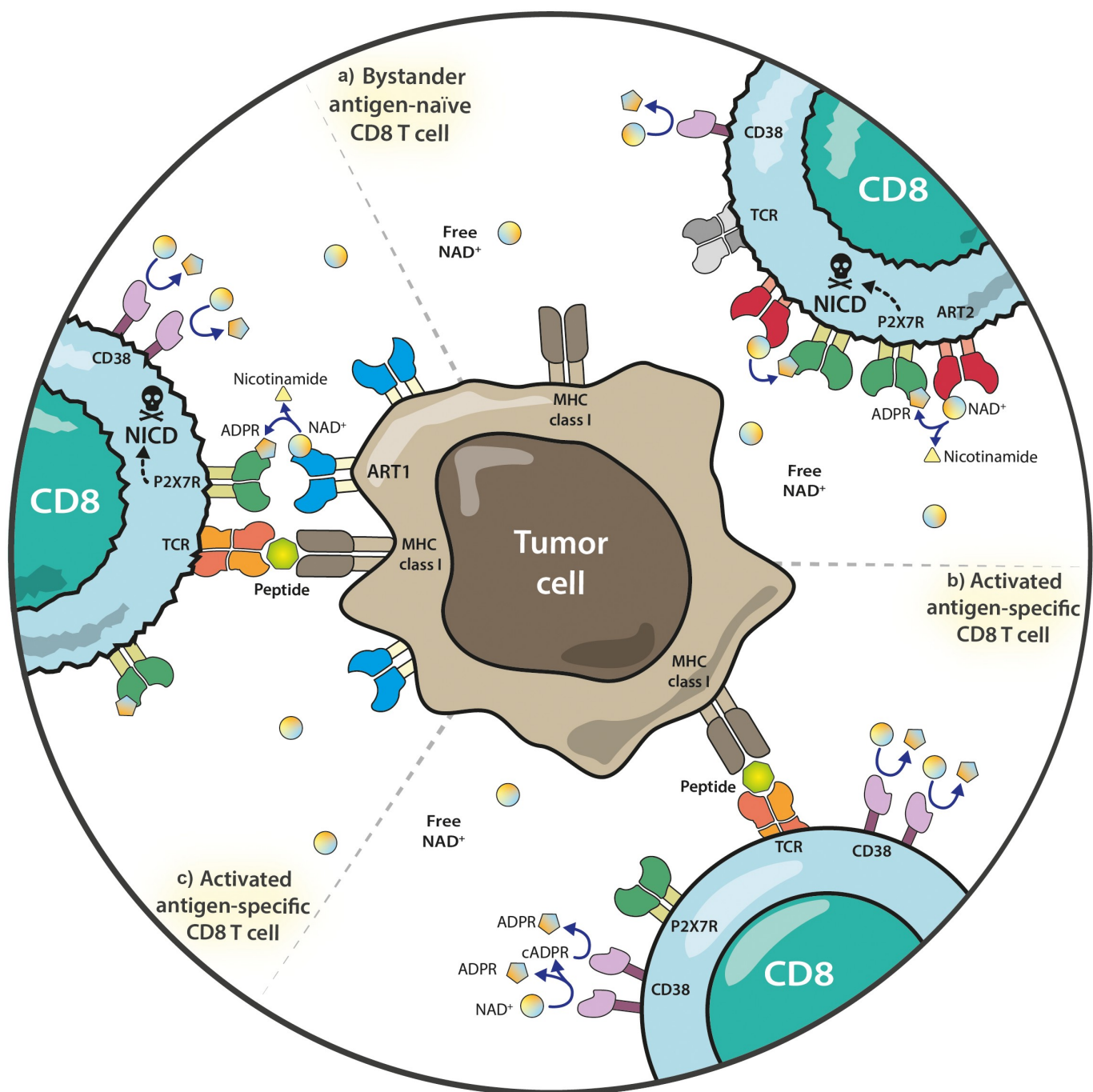


Figure 1. ART expression in tumor cells subverts immune homeostasis via NICD. NICD is a tissue immune homeostatic mechanisms that ensures local enrichment of antigen-specific T cells to promote targeted immune responses. In a tumor setting: (a) antigen-naïve intratumoral CD8 T cells are eliminated by NICD through mono-ADP-ribosylation (MARylation) of the P2X7R by ART2 in cis. (b) Activated tumor antigen-specific T cells avoid NICD by downregulating ART2 and P2X7R while upregulating the ecto-NADase CD38 thus depleting free NAD⁺ locally. (c) By expressing ART1, tumor cells MARylate the P2X7R on tumor antigen-specific CD8 T Cells in trans, thus eliminating them from the tumor microenvironment. Here, CD38 expression by CD8 T Cells remains an important cytoprotective factor against ART1-mediated NICD. NICD, NAD-induced cell death; NAD⁺, nicotinamide adenine dinucleotide; ADPR, adenosine-diphosphate-ribose; cADPR, cyclic ADPR, P2X7R, P2X7 receptor; ART1, ADP-ribosyltransferase-1; ART2, ADP-ribosyltransferase-2; TCR, T cell receptor; MHC, major histocompatibility complex.

preferentially generate long-lived central memory T cells (T_{CM}) and T_{RM} populations, important subsets for tumor immune surveillance that have been shown to be susceptible to NICD.^{3,8} Interestingly, P2X7R is also expressed in antigen-presenting dendritic cells (DC) where its activation leads to increased production of interleukin (IL)-18 in a NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3)-dependent manner, in turn activating other immune cells to produce

interferon- γ (IFN- γ) and increase tumor immunogenicity.⁹ Treatment of lung and melanoma tumor-bearing mice with a P2X7R activator potentiated the anti-tumor effect of anti-PD-1 antibody, with combined treatment leading to tumor regression and memory antitumor immune responses.¹⁰

Collectively, these data present a powerful case for the role of ART1 as a new immune checkpoint in solid tumors, whose effects are mediated by NICD of P2X7R⁺ immune cells.

P2X7R⁺ CD8 T cells and DCs appear to be critical for generating immune responses and tumor memory.^{8,9} In a subset of patients with ART1^{high} tumors, P2X7R⁺ anti-tumor memory T cell progenitors as well as DCs may be depleted through NICD, thus abrogating, at both the priming and effector level, the ability of the patient to mount effective immune responses against their cancer. This tumor defense mechanism is likely particularly engaged at times of ongoing cellular stress or cell death when NAD⁺ is released in the TME to serve as a substrate for ART1. We therefore hypothesize that combining an anti-ART1 therapeutic with cytotoxic therapies, such as chemotherapy or radiotherapy, will be particularly beneficial. Because ART1 blockade increased the proportion of tumor infiltrating Ki67⁺/PD-1⁺ CD8 T cells, we also envision a role for combining an anti-ART1 therapeutic with PD-1/programmed death-ligand 1 (PD-L1) inhibitors in ART1^{high} tumors. In addition, our findings have implications for strategies targeting P2X7R or CD38. By preventing NICD of P2X7R⁺ immune cells, an anti-ART1 therapeutic might work synergistically with P2X7R agonists to enhance the anti-tumor immune response. Conversely, the treatment of ART1^{high} tumors with anti-CD38 antibodies might have the detrimental effect of preserving NAD⁺ in the TME to serve as a substrate for ART1-induced MARYlation and NICD of immune cells. This effect may be exacerbated in patients whose T cells are primed against their tumor, such as following immunotherapy. Indeed, clinical lung cancer trials utilizing anti-CD38 antibodies combined with PD-1/PD-L1 inhibitors have to date been unsuccessful.

In conclusion, while more attention has historically been given to poly-ADP-ribosyltransferases as cancer therapeutics, mono-ADP-ribosyltransferases are increasingly recognized for playing a role in cancer progression. MARYlation may significantly alter cell signaling pathways in tumor cells through post-translational modification of key regulatory proteins. Our recent findings show that MARYlation also regulates the anti-tumor immune response by modifying cell surface proteins in immune cells in trans. As such, mono-ADP-ribosyltransferases are bona fide therapeutic targets. As new techniques are developed to better identify targets of MARYlation, we expect that interest in ART family members and biology will continue to grow.

Disclosure statement

B.M.S.: Consulting/Advisory Boards: AstraZeneca, Pfizer, Medtronic, Genentech, Bristol Myers Squibb, Galvanize Therapeutics, Flame Biosciences. Research Support: Bristol Myers Squibb, Bristol Myers Squibb Foundation, Lungevity, Mark Foundation for Cancer Research. Board/Scientific Advisory Board: Lung Cancer Research Foundation, Lungevity. E.W., S.M. and R.M.S. have declared no competing interests.

Funding

Funding was provided from the Lung Cancer Research Foundation and the Department of Defense CDMRP Lung Cancer Research Program.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

References

1. Demeny MA, Virag L. The PARP enzyme family and the hallmarks of cancer part 1. *Cell Intrinsic Hallmarks Cancers* (Basel). 2021;13(9):2042.
2. Di Girolamo M, Fabrizio G. Overview of the mammalian ADP-ribosyl-transferases clostridia toxin-like (ARTCs) family. *Biochem Pharmacol.* 2019;167:86–96. doi:10.1016/j.bcp.2019.07.004.
3. Stark R, Wesselink TH, Behr FM, Kragten NAM, Arens R, Koch-Nolte F, van Gisbergen KPJM, van Lier RAW. T RM maintenance is regulated by tissue damage via P2RX7. *Sci Immunol.* 2018;3(30). doi:10.1126/sciimmunol.aau1022.
4. Adriouch S, Hubert S, Pechberty S, Koch-Nolte F, Haag F, Seman M. NAD⁺ released during inflammation participates in T cell homeostasis by inducing ART2-mediated death of naive T cells in vivo. *J Immunol.* 2007;179(1):186–194. doi:10.4049/jimmunol.179.1.186.
5. Wennerberg E, Mukherjee S, Spada S, Hung C, Agrusa CJ, Chen C, Valeta-Magara A, Rudqvist NP, Van Nest SJ, Kamel MK, et al. Expression of the mono-ADP-ribosyltransferase ART1 by tumor cells mediates immune resistance in non-small cell lung cancer. *Sci Transl Med.* 2022;14(636):eabe8195. doi:10.1126/scitranslmed.abe8195.
6. Di Virgilio F, Dal Ben D, Sarti AC, Giuliani AL, Falzoni S. The P2X7 Receptor in Infection and Inflammation. *Immunity.* 2017;47(1):15–31. doi:10.1016/j.immuni.2017.06.020.
7. Adinolfi E, Capece M, Franceschini A, Falzoni S, Giuliani AL, Rotondo A, Sarti AC, Bonora M, Syberg S, Corigliano D, et al. Accelerated tumor progression in mice lacking the ATP receptor P2X7. *Cancer Res.* 2015;75(4):635–644. doi:10.1158/0008-5472.CAN-14-1259.
8. Vardam-Kaur T, van Dijk S, Peng C, Wanhainen KM, Jameson SC, Borges da Silva H. The extracellular ATP receptor P2RX7 imprints a promemory transcriptional signature in effector CD8 + T cells. *J Immunol.* 2022;208(7):1686–1699. doi:10.4049/jimmunol.2100555.
9. Aymeric L, Apetoh L, Ghiringhelli F, Tesniere A, Martins I, Kroemer G, Smyth MJ, Zitvogel L. Tumor cell death and ATP release prime dendritic cells and efficient anticancer immunity. *Cancer Res.* 2010;70(3):855–858. doi:10.1158/0008-5472.CAN-09-3566.
10. Douguet L, Janho Dit Hreich S, Benzaquen J, Seguin L, Juhel T, Dezitter X, Duranton C, Ryffel B, Kanellopoulos J, Delarasse C, et al. A small-molecule P2RX7 activator promotes anti-tumor immune responses and sensitizes lung tumor to immunotherapy. *Nat Commun.* 2021;12(1):653. doi:10.1038/s41467-021-20912-2.