Background Intracellular nicotinamide phosphoribosyltransferase (iNAMPT) serves as the enzyme that regulates NAD salvage pathway. Additionally, NAMPT can be found in the extracellular space, referred to as extracellular NAMPT (eNAMPT), where it behaves as a cytokine. It is worth noting that eNAMPT has also been identified as an oncogenic factor in breast cancer (BC), with its increased levels being associated with tumour size and histological grading. However, the specific role of eNAMPT as a cytokine in the progression of BC remains less understood. Therefore, the objective of our study is to investigate the potential of eNAMPT as a cytokine in an in vivo model of triple negative mammary carcinoma (TNBC).

Methods We used female BALB/c mice injected with 4T1, assessing tumoural size, spleen weight and number of metastases. We administered anti-eNAMPT neutralizing antibody two times a week and we sacrificed the mice after 28 days. Harvested tumours were analysed by histopathology, flow cytometry, Western Blot, immunohistochemistry, immunofluorescence. We performed RNA sequencing to define tumour characteristics (isolating tumour infiltrating lymphocytes and tumoural cells from primary tumours) and to explore the molecular mechanisms behind the observed phenotype. Moreover, we dissected the functional relationship between T cells and tumoural cells, using 3D co-cultures.

Results Interestingly, neutralizing eNAMPT with C269 resulted in a reduction in mammary carcinoma growth. This effect was attributed to a decreased expression of genes involved in angiogenesis, hypoxia, cell adhesion, and immunosuppressive phenotype, while there was an increase in genes related to epithelial cell differentiation and apoptosis. Surprisingly, when examining the role of eNAMPT in vitro, we did not observe any significant impact on proliferation, suggesting that the observed in vivo effect is likely mediated by the tumour microenvironment. We did not appreciate any changes in the frequency of myeloid immune populations within the tumour. However, there was a reduction in the frequency of immunosuppressive PD-1+ Treg cells in C269-treated mice, accompanied by an increase in CD8+ IFNγ+ cells. Additionally, the levels of PD-1 and PD-L1 in tumours were reduced upon treatment with C269. Furthermore, we found that C269 enhanced the ability of CD4 and CD8 cells to infiltrate 4T1 spheroid model, demonstrating its potential to promote immune cell infiltration into tumour cells. These findings suggest that neutralizing eNAMPT with C269 can impede mammary carcinoma growth by enhancing immune cell infiltration into tumour cells.

Conclusions These studies indicate for the first time eNAMPT as a novel immunotherapeutic target for triple negative breast cancer.

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Ethics Approval Animal care was in compliance with Italian regulations and was authorised by the Ministry of Health (120/2018 DB064.30 of 27/03/2018) and conducted under ARRIVE1 reporting guidelines.