89 NON-INVASIVE IMAGING OF TUMOR-INFILTRATING B LYMPHOCYTES IN A TRIPLE NEGATIVE BREAST CANCER MODEL USING ANTI-CD20 IMMUNOPET

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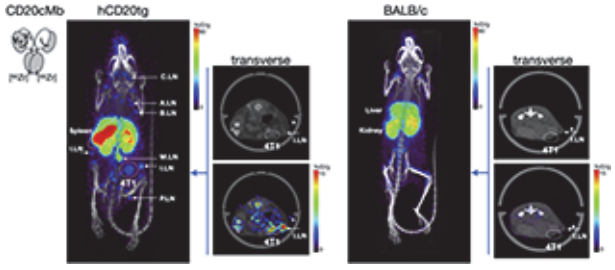
Background Tumor-infiltrating B lymphocytes (TIL-Bs) and tertiary lymphoid structures (TLS) play critical roles in anti-tumor immunity. However, their predictive and prognostic value remains unclear. This study aimed to develop anti-human CD20 immunoPET imaging as a tool to assess the potential of TIL-Bs and TLS as predictors of therapy response and evaluate the changes in the B-cell compartment as an integrated readout of therapy response. In a proof-of-principle study we employed a radiolabeled CD20 antibody fragment (^{89}Zr-CD20cMb) to non-invasively monitor CD20-positive B cells in a syngeneic model of triple-negative breast cancer (4T1) in human CD20 transgenic mice (hCD20tg).

Methods Tumors were orthotopically implanted into the mammary fat pad, and mice were treated with intratumoral injections of CpG-ODN 1826 or left untreated. Antigen negative BALB/c mice were used as control. The CD20cMb was radiolabeled with zirconium-89 (^{89}Zr) with high efficiency. Serial immunoPET/CT scans were conducted at 4, 24, and 48 h post injection of ^{89}Zr-CD20cMb. Ex vivo biodistribution was performed after the last scan. Presence of B cells (B220) and T cells (CD3) in tumors and lymphoid tissues was confirmed by IHC.

Results The results demonstrated successful visualization of TIL-Bs using anti-CD20 immunoPET (figure 1). The immunoPET scans showed tracer uptake in lymphoid tissues as early as 4 hours post-injection, with high uptake in the spleen of hCD20tg (156 ± 26 %ID/g) that was not observed in the spleen of BALB/c (14.6 ± 1.6 %ID/g). Tracer signal in 4T1 tumors in hCD20tg mice indicated the presence of TIL-Bs and was significantly higher than in antigen-negative control mice (8.7 ± 2.6 vs 4.7 ± 1.6 %ID/g). Furthermore, higher uptake was observed in the draining inguinal lymph nodes, indicating a weak but detectable anti-tumor immune response. Treatment with intratumoral CpG resulted in tumor growth inhibition and increased signal in the spleen (splenomegaly) and draining lymph nodes, indicating systemic immune activation. The increased accumulation of B cells in the tumor tissue was confirmed through IHC.

Conclusions In conclusion, anti-CD20 immunoPET effectively visualized the B-cell compartment and tumor-infiltrating B cells in a syngeneic breast cancer model using human CD20 transgenic mice. This model enables monitoring changes in B-cell distribution over time and in response to immunotherapy.

Ethics Approval All procedures performed in studies involving animals were in accordance with the ethical standards of the City of Hope Animal Research Committee and were approved by the Institutional Animal Care and Use Committee.



Abstract 89 Figure 1 Anti-CD20 immunoPET of TIL-Bs. ^{89}Zr-CD20cMb in hCD20 transgenic mice shows antigen-specific uptake in lymphoid tissues (spleen, lymph nodes) that not seen in wild-type BALB/c mice. Signal in the orthotopically implanted 4T1 TNBC tumor and higher signal in the draining inguinal lymph node indicate an anti-tumor immune response and the presence of TII-Bs. Tumors and lymph nodes in the control mice are not visible confirming specificity. Lymph nodes: C.LN. cervical, A-LN. axillary, B.LN. brachial, M.LN. mesenteric, I.LN. inguinal, PLN. popliteal.

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