INVESTIGATING THE ROLE OF ATYPICAL B CELLS IN RESPONSE TO BCG IN NON-MUSCLE INVASIVE BLADDER CANCER

Priyanka Yolmo[1,2], Sadaf Rahimi[1,2], Gwenaelle Conseil[1,2], Kartik Sachdeva[1,2], D. Robert Siemens[4] and Madhuri Koti[1,2,3,4]

[1]Queen's Cancer Research Institute,[2]Department of Biomedical and Molecular Sciences, [3]Department of Urology and [4]Department of Obstetrics and Gynecology, Queen's University, Kingston, ON, Canada

Background: Non-muscle invasive bladder cancer (NMIBC) comprises 75% of bladder cancer (BC) cases being diagnosed worldwide. Intravesical bacilllus Calmette-Guerin (BCG) immunotherapy is the gold standard treatment for intermediate and high-risk NMIBC. Despite its proven efficacy, over 50% patients receiving BCG immunotherapy experience early recurrence or progression and 25-40% show progression to muscle invasive disease. Increased intra-tumoral infiltration in pre-treatment tumors has been associated with poor outcomes post BCG therapy. In our recent study, we highlighted high intra-tumoral B cell density to be associated with early recurrence and progression of NMIBC. Since, atypical B cells (ABCs), a subset of B cells are known to expand with biological aging, repetitive immunization and/or in autoimmune diseases, we hypothesized that ABCs are recruited to the bladder mucosa during repeated BCG instillation in the induction phase of treatment and dampen the local anti-tumor immunity leading to poor response to BCG.

Methods: The N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) carcinogen induced murine model of BC was used to investigate the role of B cells in mediating BCG response. Female and male mice exposed to BBN were treated with intravesical BCG immunotherapy with or without B cell depletion. Systemic and local immune profiling was done using multispectral flow cytometry and multiplex immunofluorescence. Plasma cytokine levels were analyzed using multiplex cytokine profiling.

Results: Increased infiltration of B cells and expansion of ABCs systemically and locally (bladder) was observed after repeated BCG instillations. *In vivo* depletion of B cells during BCG treatment in mice exposed to BBN showed enhanced recovery of the urothelium with high CD11b+ myeloid cell infiltration. Depletion of B cells during BCG treatment also altered the frequency of T cell subsets and depicted elevated levels of plasma Th1 and Th2 cytokines.

Conclusion: Results from *in vivo* studies demonstrated a potential role of ABCs in mediating poor response to BCG. Therapeutic targeting of ABC associated markers could be a novel approach for treatment of NMIBC patients.