ONCOTHERAD IMMUNOTHERAPY INDUCES TOLL-LIKE RECEPTOR 4-MEDIATED INTERFERON SIGNALING PATHWAY AND LEADS TO FORMATION OF PRIMARY LYMPHOID FOLLICLES IN BACILLUS CALMETTE–GUERIN-UNRESPONSIVE NON–MUSCLE INVASIVE BLADDER CANCER

João Carlos Cardoso Alonso[1,2], Ianny Brum Reis, Bianca Ribeiro de Souza Sasaki[1], Adriano Angelo Cintra[1,2], Nelson Durán[1], Athanase Billis[3], Wagner José Fávaro[1]

LABORATORY OF UROGENITAL CARCINOGENESIS AND IMMUNOTHERAPY (LCURGIM)

[1]Laboratory of Urogenital Carcinogenesis and Immunotherapy, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil;
[2]Paulínia Municipal Hospital, São Paulo, Brazil;
[3]Department of Pathology, School of Medicine, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil.

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INTRODUCTION AND OBJECTIVES

Primary lymphoid follicles (PLFs) are backbone of the formation of follicular dendritic networks, which are the main important questions for germinal center responses, during which affinity maturation creates applications optimized in adaptive immune responses. Toll-like receptors (TLRs) expression by myeloid lineage cells provides a bridge between the innate and adaptive immune responses in dendritic cells and cells of lineage of macrophages-monocytes that express TLRs, improving the antigen-presenting cell function and the release of essential cytokines and cytokines for activation of $CD8^+$ and $CD4^+$ T cells. The contribution of PLFs associated responses is poorly understood in bladder cancer. The new modalities for treating patients with non-muscle invasive bladder cancer (NMIBC) for whom Bacillus Calmette-Guerin (BCG) has failed or is contraindicated are recently increasing due to the development of new drugs.

This study characterized the mechanisms of action of OncoTherad immunotherapy based on activation of TLR4-mediated interferon signaling pathway and formation of TLS in bladder tissues from BCG-unresponsive NMIBC patients.

METHODS

We conducted a prospective, single-center (Municipal Hospital of Paulinia, São Paulo, Brazil), single-arm phase I/ II study of OncoTherad immunotherapy in 29 (18 male, 11 female) patients with BCG-unresponsive NMIBC (≥ 1 previous course of BCG intravesical therapy). The schedule was initiated with weekly intravesical (120 mg/mL) and intramuscular (25 mg/mL) OncoTherad treatment for 6 weeks, followed by one every other week application for 3 months and, one monthly application until the end of the treatment (24 months).

RESULTS

OncoTherad treatment showed pathological complete rates of 89,6% (26/29) at 24-months follow-up. Also, OncoTherad immunotherapy led to formation of PLFs in most patients (34.5%) after 24-months follow-up. TLR4, TRIF, IRF-3, IFN-**γ** and iNOS immunoreactivities were significantly intense in the regions of PLFs present in the bladder samples from patients treated with OncoTherad after 24-months follow-up in relation to bladder tissue samples that preceded OncoTherad treatment.

Table 1: Average immunohistochemistry intensity for different antigens in the urinary bladder before and after treatment with OncoTherad.

Antigens	Groups	
	Before OncoTherad (n=29)	After OncoTherad (n=29)
TLR2	1 (9.8%)	3 (88.5%)*
TLR4	1 (11.9%)	3 (95.2%)*
TRIF	1 (17.4%)	3 (87.6%)*
IRF-3	1 (12.1%)	3 (92.3%)*
IFN-γ	1 (21.2%)	3 (94.8%)*
iNOS	1 (8.6%)	3 (96.9%)*

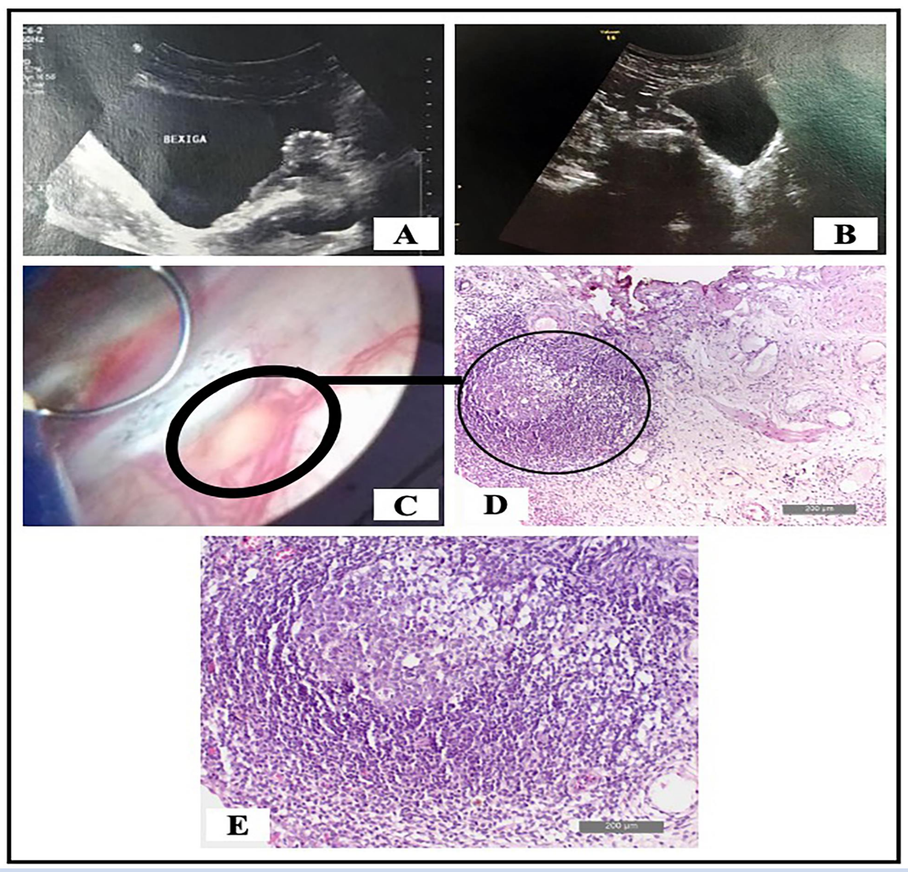

Figure 1: Ultrasonography, Cystoscopy and Photomicrographs representative of the urinary bladder. Before treatment with OncoTherad: (A) ultrasound image revealing a hyperechoic lesion, measuring 3.1 x 1.44 cm on the bladder floor. After 24-month follow-up with OncoTherad: (B) ultrasound image revealing a bladder with thin and thin walls, with anechoic and homogeneous content; (C) cystoscopic image revealing a flat lesion (circle) <3 cm in the bladder floor, characterized by chronic follicular cystitis; (D), (E) Photomicrographs of the same flat lesion found in cystoscopy: primary lymphoid follicle (circle) characterized by the presence of a germinal center containing lymphocytes, in addition to centrocytes (small and cleaved), centroblasts (large and nucleoli) and macrophages.

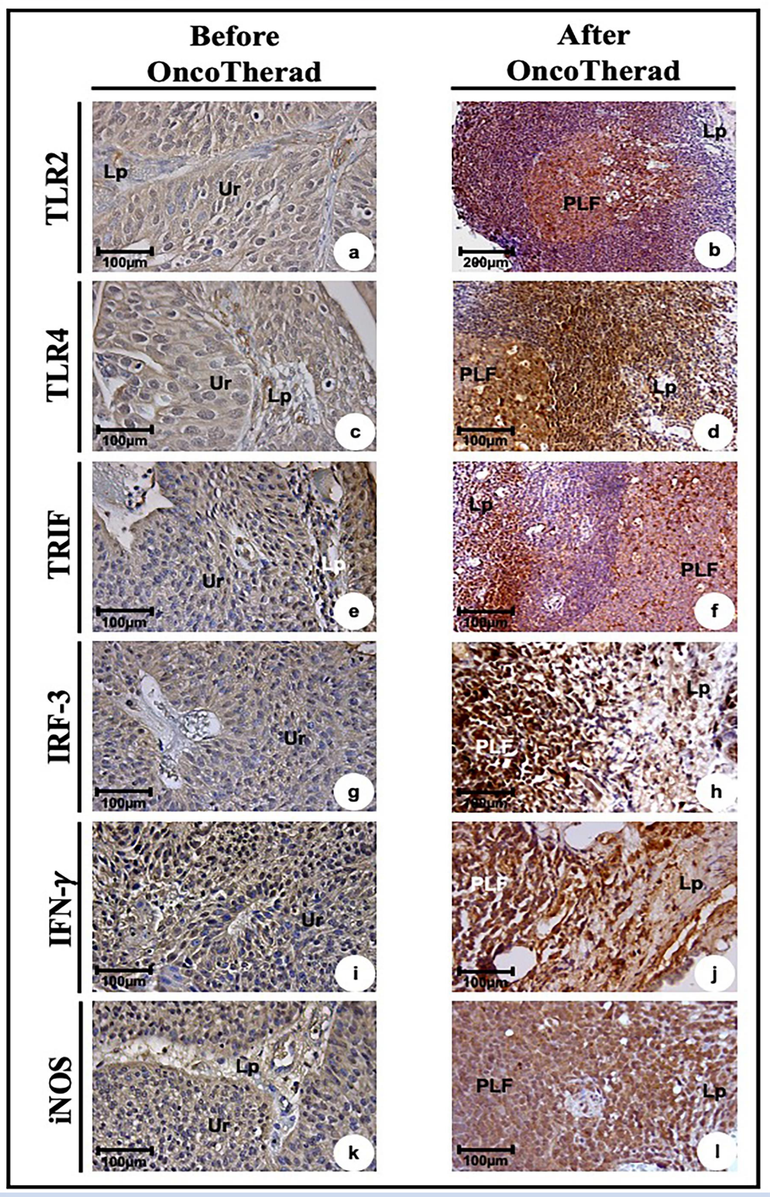

Figure 2: Representative immunohistochemistry of the urinary bladder before treatment with OncoTherad (a, c, e, g, i, k) and after 24-month follow-up with OncoTherad (b, d, f, h, j, l) . (a), (b) Immunohistochemistry for TLR2 (a, b), TLR4 (c, d), TRIF (e, f), IRF-3 (g, h), IFN-γ (i, j) and iNOS (k, l) in the urothelium and PLF region. a - l: Lp - lamina propria, PLF - primary lymphoid follicle, Ur - urothelium.

CONCLUSION

In conclusion, this study demonstrated an important effect of activation of the TLR4 signaling pathway triggered by OncoTherad in the formation and organization of PLFs, which may be related to antitumor and immunoprotective effects from this immunotherapy in bladder tissue.

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