ONCOTHERAD IMMUNOTHERAPY AND PLATELET RICH PLASMA IN THE BLADDER CANCER TREATMENT: ASSESSMENT OF THE CYTOTOXIC RESPONSE AND CANCER PROGRESSION BIOMARKERS

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Background

- The type I and II **interferon** (**IFN**) response is related to the activation of **tank-binding kinase 1** (**TBK1**) through **Toll-like receptors** (**TLR**).
- IFNs promote **CD8+T cells** expansion and activation, increasing **immune surveillance**.
- Recently, **CX3C chemokine receptor 1** (**CX3CR1**) was discovered to be a **T cell differentiation marker**.
- **Regulatory T cells** (**Tregs**), identified by **FOXP3** marker, allow **immune tolerance**.

Objective

Evaluate the effects of **OncoTherad** nanoimmunotherapy associated with **Platelet Rich Plasma** (**PRP**) on **cytotoxic activity** (TBK1, CX3CR1, IL1-β), **Tregs FOXP3+**, and **cancer progression biomarkers** (VEGF and IGF-1) in a **non-muscle invasive bladder cancer** (NMIBC) mouse model.

Methods

- 35 **C57BL/6J mice** were induced with **N-ethyl-N-nitrosourea** carcinogen (50 mg/ml);
- The **intravesical doses** (0.1 ml) were instilled once a week for 6 weeks;
- Bladder immunohistochemistry was analyzed in two ways: **Total Immunoreactivity** (antigen positive cells) and **intensity of antigen immunoreaction** (absent, weak, moderate, or strong).

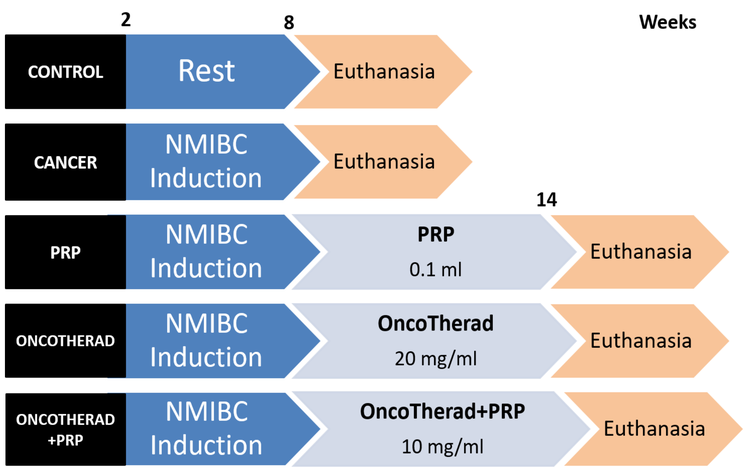

Figure 1. Experimental Protocol.

Results

- **OncoTherad** and **OncoTherad+PRP** treatments **increased TBK1** immunoreactivity and the immunoreaction intensity;
- There was an **increase** in the number of **CX3CR1+ cells** and the immunostaining intensity after **OncoTherad** treatment;
- Both total immunoreactivity and **IL1-β** intensity **decreased** in the **Cancer** group;
- **OncoTherad+PRP** group showed a **higher** percentage of **IL1-β** positive cells and a **stronger** immunoreaction;
- **OncoTherad** and **OncoTherad+PRP decreased** the percentage of **FOXP3+ cells** and the reaction intensity;
- **PRP** alone or with **OncoTherad did not increase VEGF** and **IGF-1** growth factors.

Conclusions

OncoTherad alone or with **PRP modulated** the NMIBC microenvironment to a **cytotoxic profile** correlated with the **IL1-β increase** by stimulating immune pathways for IFNγ production and consequent **CD8+T cell activation** and **Tregs reduction**. In addition, **PRP did not trigger carcinogenic effects** in this NMIBC model.

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Main findings:

The **increase** of **TBK1** by **OncoTherad** and **OncoTherad+PRP** confirms the mechanism of action through **TLR4 pathway**;

The **stimulation** of **TLR4**-mediated **IFNγ production** is related to the **higher** number of **CX3CR1+ cells** in the **OncoTherad** group, since **IFNγ** is central to **CD8+T cell activation**;

This **polarization** to a **cytotoxic** microenvironment is consistent with the **FOXP3+ Tregs reduction** and the **IL-1β increase** (key cytokine during pyroptosis or inflammatory cell death);

The **growth factors** related to tumor progression were **not increased** by **PRP**.

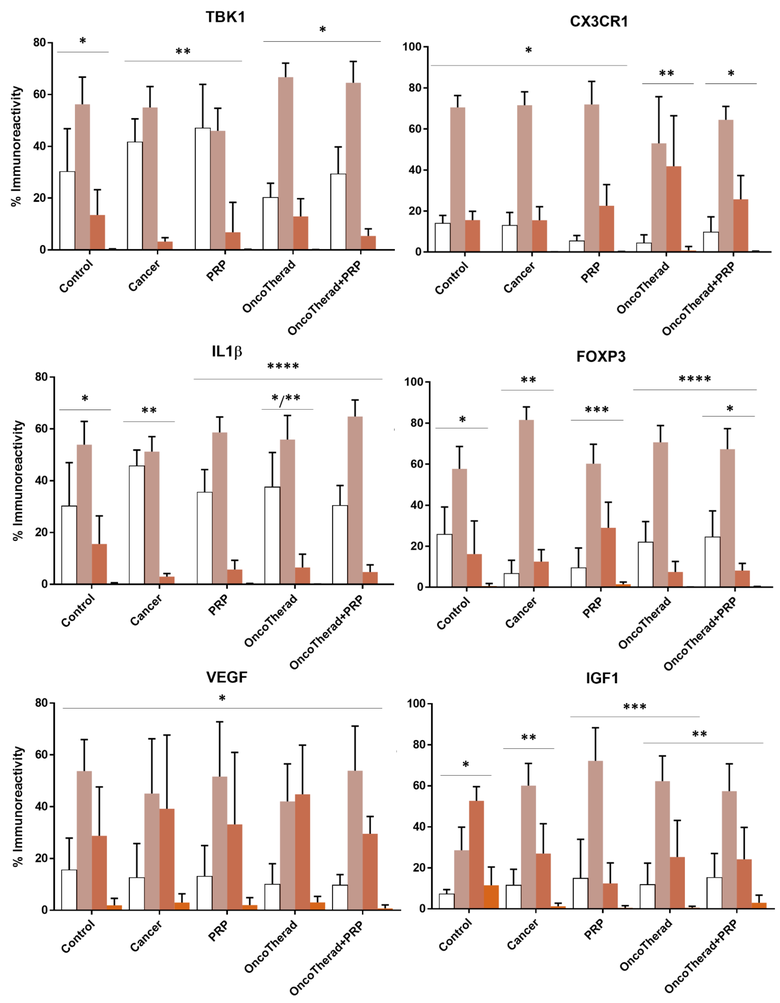

Figure 2. Immunoreactivities (%) in each intensity category by group for the antigens. */**/***/**** Different symbols indicate a significant difference ($p<0.05$) between the groups. ANOVA, Tukey's test (n=5 sections/ animal/group).

Table 1. Total Immunoreactivity of the antigens in urinary bladder urothelium (%).

	Experimental Groups				
Antigens	**Control**	**Cancer**	**PRP**	**OncoTherad**	**OncoTherad+PRP**
TBK1	69.72 ac	58.32 ab	52.97 b	79.76 c	70.63 ac
CX3CR1	86.06 a	87.09 a	94.57 bc	95.58 b	90.29 ac
IL1-β	69.75 a	54.26 b	64.45 ab	62.44 ab	69.57 a
FOXP3	74.37 a	93.41 b	90.55 b	78.08 a	75.58 a
VEGF	84.45 a	87.35 a	86.87 a	89.92 a	90.29 a
IGF-1	92.77 a	88.47 a	85.14 a	88.16 a	84.71 a

Values are equivalent to the medians of the percentage of positive urothelial cells for antigens (n=5 sections/animal/group). Kruskal-Wallis, Student-Newman-Keuls test. In the same line, values followed by different letters indicate a significant difference between groups ($p<0.05$).

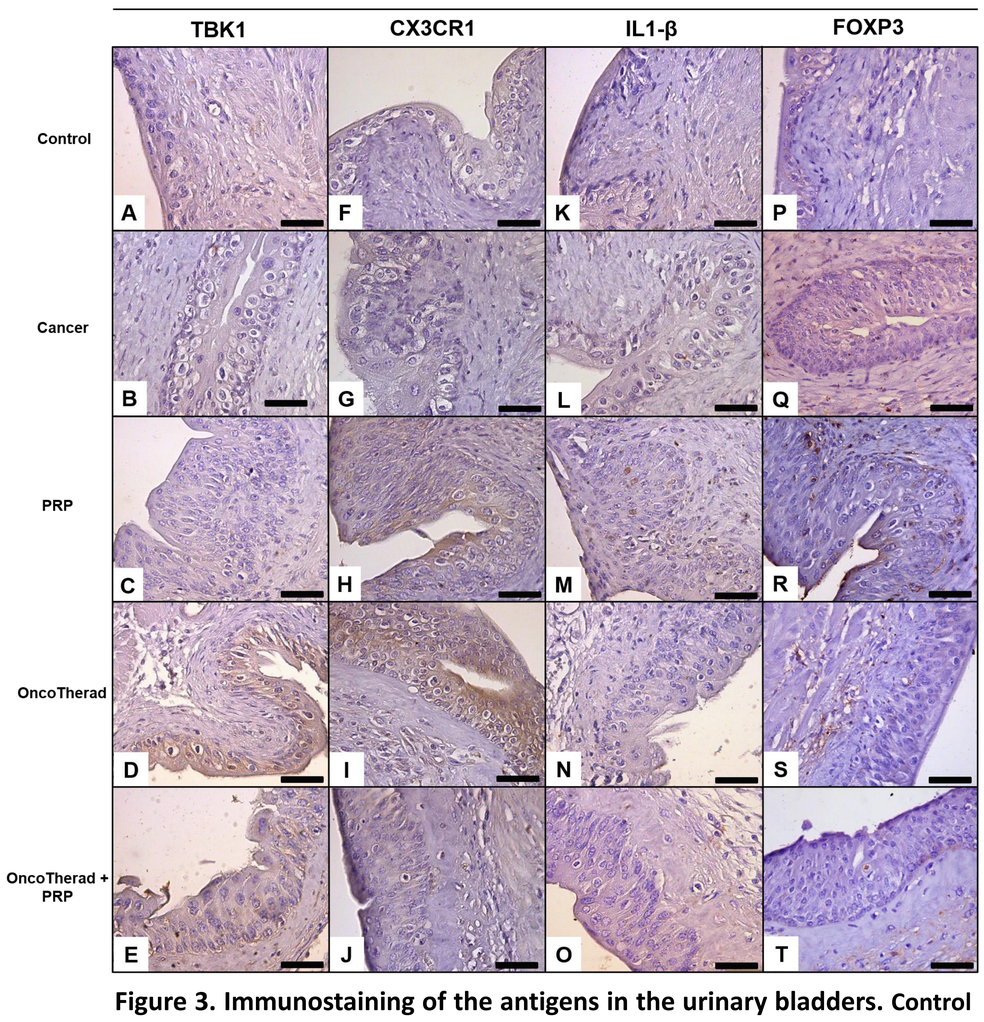

Figure 3. Immunostaining of the antigens in the urinary bladders. Control (healthy urothelium), **Cancer** (urothelial carcinoma in situ or pTis), **PRP** (pTa non-invasive urothelial carcinoma), **OncoTherad** (flat hyperplasia), and **OncoTherad+PRP** (flat hyperplasia). Bars = 50 µm.

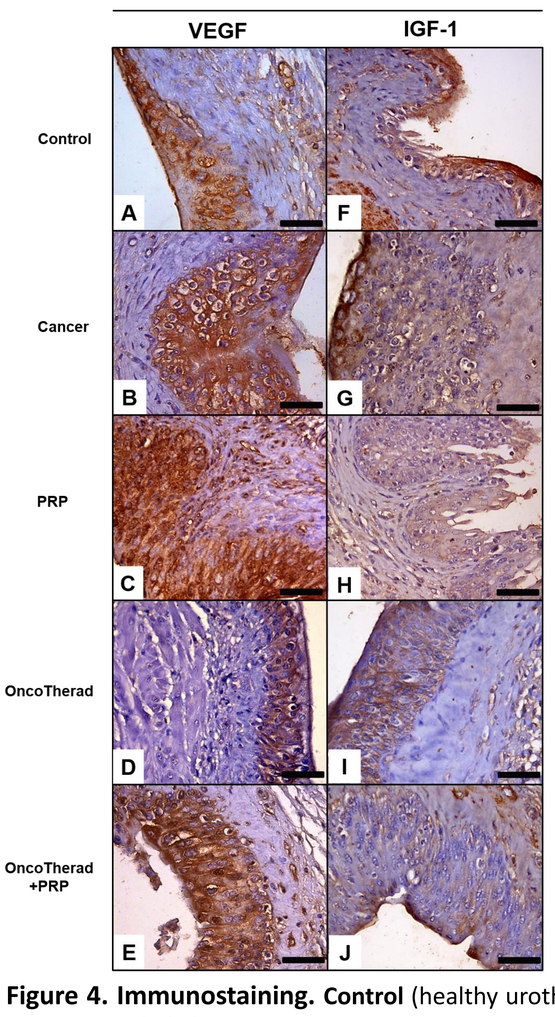

Figure 4. Immunostaining. Control (healthy urothelium), **Cancer** (urothelial carcinoma in situ or pTis), **PRP** (pTa non-invasive urothelial carcinoma), **Oncotherad** and **OncoTherad+PRP** (flat hyperplasia). Bars = 50 µm.