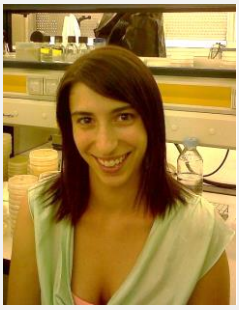
ImmunoTools *special* Award 2014



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Immune response triggered by a new immunotherapeutic option for bladder cancer treatment

Bladder cancer (BC) is one of the most common cancers around the world. The numbers are impressive: over 330300 new cases were diagnosed and 123043 deaths are estimated to be caused by bladder cancer worldwide in 2012. Between 75-85% of BC patients have non-muscle invasive disease at diagnosing, being transurethral resection of the tumor the first step for treatment. Subsequently, intravesical instillation of the attenuated *Mycobacterium bovis* BCG is the most efficacious treatment for avoid recurrences.

On the one hand, live BCG exerts a direct antitumor activity by inhibiting cellular proliferation and, on the other hand, an indirect antitumor effect involving the stimulation of a robust immune response helping the host immune system to eradicate cancer cells. Among others, BCG triggers the induction of cytokines production which result in the recruitment of different immune cells, including macrophages, dendritic cells, natural killer cells and afterwards B cells, suppressor and helper T cells.

Despite BCG benefits, this immunotherapy treatment is not perfect. BCG causes from non-severe toxicity associated problems to more severe adverse events, including BCG infections. Although different approaches for entirely replacing live BCG have been studied (ie. heat-killed BCG), to date, no strategy had been shown to be as effective as live BCG. Our research is focus on improving the current BCG treatment.

In this way, we have recently demonstrated that killed but metabolically active BCG (γ-irradiated BCG) could be a proper alternative to live BCG (Secanella-Fandos et al., 2014). Gamma-irradiated BCG is safe because the bacteria is not viable, however the expression, presentation, and secretion of some antigens outside the cell is preserved. In in vitro experiments we have demonstrated a higher direct antitumor

capacity of γ-irradiated BCG than heat-killed BCG, inhibiting BC cells growth and triggering the production of cytokines by BC cells. Moreover, γ-irradiated BCG preserves the BCG capacity to activate peripheral blood mononuclear cells which in turns are able to inhibit BC cells growth. Furthermore, we have developed a new formulation based on an oil-in-water emulsion of BCG, which have also demonstrated in in vitro experiments to improve the antitumor efficacy of the mycobacterium (Julián et al, 2013).

To further investigate the action of these two improved immunotherapeutic options, we are currently using an established mouse orthotopic bladder model which mimics the human disease. We are first evaluating if mice bearing tumors treated with g-irradiated BCG formulated in oil-in-water emulsion survive longer or similarly to mice bearing tumors treated with live BCG. Later, the ImmunoTools reagents will be essential to enable us to go deeper into the mechanism of action of this novel immunotherapeutic treatment. We aim to determine the systemic and local immune response that occurs when mice bearing bladder tumors are treated with γ-irradiated BCG compared to live BCG. Thus, after inducing BC to the animals, four weekly treatments will be administered through the urethra. The day after the last dose, all animals will be sacrificed and bladders will be collected for studying the local response that is taking place by using flow cytometry technology. We will analyze the immune cells that are taking part in the generated immune response. Detailed determination of the systemic and local response to the treatment *in vivo* is of great importance, as it may offer basic knowledge for the design of safer immunotherapy strategies in bladder cancer patients.

References:

- Secanella-Fandos S, **Noguera-Ortega E**, Olivares F, Luquin M, and **Julián E**. (2013) Killed But Metabolically Active Mycobacterium bovis BCG retains the antitumor ability of live BCG. The Journal of Urology. In press.
- **Julián E**, Luquin M, and **Noguera-Ortega E**. Patent application.