Abstract # 2755

UBLITUXIMAB (TGTX-1101), A NOVEL THIRD-GENERATION ANTI-CD20 ANTIBODY DEMONSTRATES ENHANCED ANTITUMOR ACTIVITY COMPARED TO RITUXIMAB IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMAS MURINE MODELS

Donnou Sabrina[1,2], Ben Abdelwahed-Bagga Rym[1], Cosette Jérémie[1], Ouakrim Hanane[1], Crozet Lucile[1], Jacquet Alexandra[3], Fridman Wolf Herman[1], Sautès-Fridman Catherine[1], Sportelli Peter[4*], Urbain Rémi[3], and Fisson Sylvain[1,2]

[1]Cordeliers Research Center, INSERM UMRS872, immune microenvironment and tumors group / Pierre and Marie Curie-Paris 6 University / Paris-Descartes University, Paris, France; [2]Généthon, Evry / INSERM UMRS951 / University of Evry Val d'Essonne, UMRS 951, Evry, France; [3]LFB Biotechnologies, Les Ulis, France; [4]TG Therapeutics, New York, USA; *corresponding author: ps@tgtxinc.com

BACKGROUND

Primary cerebral lymphomas (PCL), related to the systemic diffuse large B-cell lymphoma family, are highly aggressive tumors with poor prognosis and no specific standardized therapy. Despite good results obtained with high dose chemotherapy, many patients relapse and new therapeutic strategies are needed. PCLs are characterized by the presence of CD20$^+$ lymphomatous B-cells and as such are suitable for therapy with anti human CD20 antibodies. In this study, we evaluated the efficiency of ublituximab, a promising glycoengineered anti-hCD20 monoclonal antibody that displays a high affinity for FcgRIIIa (CD16) receptors.

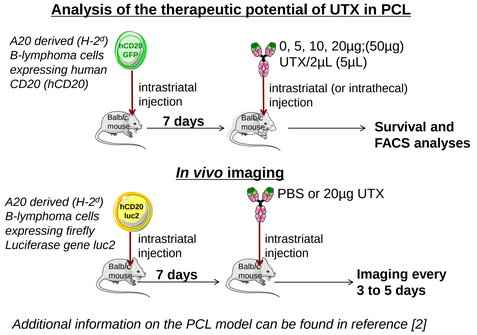
UBLITUXIMAB (UTX)

Ublituximab, a next generation anti-CD20 antibody currently in clinical development, is characterized by a specific glycosylation pattern containing a high percentage of non-fucosylated antibody molecules at the Fc site. This specific pattern of glycosylation increases the affinity of antibodies for human FcγRIIIa (CD16), resulting in an increased antibody dependent cell-mediated cytotoxicity (ADCC) by human FcγRIIIa-expressing effector cells.

RESULTS

Anti-Tumor Effect of Ublituximab in a Primary Cerebral Lymphoma (PCL) Model

Experimental Setting



Additional information on the PCL model can be found in reference [2]

Dose-Response After Intratumoral Injection

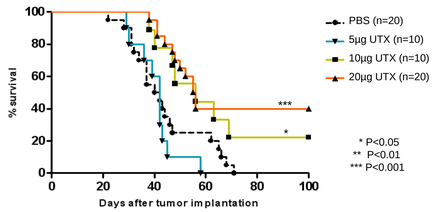


Figure 1: Kaplan-Meier survival analysis of mice inoculated intracerebrally with A20.IIA-GFP-hCD20 tumor cells and treated 7 days later with a single injection of PBS, 5µg, 10µg or 20µg of anti-hCD20 ublituximab directly into the tumor bed. Results represent a pool of 4 independent experiments.

- PBS vs. ublituximab 20µg: p=0.0006
- PBS vs. ublituximab 10µg: p=0.033
- PBS vs. ublituximab 5µg: no statistical difference
- PBS vs. isotypic control: no statistical difference (not shown) *(Log-rank test)*

T-cells Infiltration After Treatment

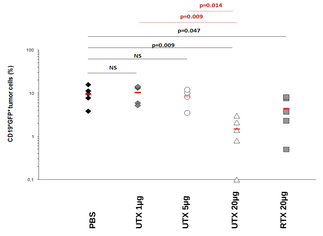


Figure 2: Flow cytometric analysis of brains inoculated with A20.IIA-GFP-hCD20 tumor cells and treated 7 days later with PBS, 1µg, 5µg or 20µg of anti-hCD20 ublituximab or 20µg of anti-hCD20 rituximab. Analyses were performed 7 days after the therapeutic administration.

In Vivo Imaging of Tumor Elimination

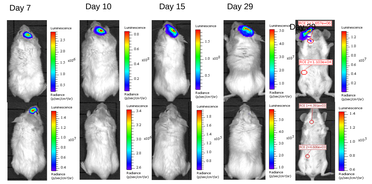


Figure 3: Mice were inoculated intracerebrally with A20.IIA-GFP-hCD20-luc2 tumor cells and were either treated or not treated 7 days later with 20µg of anti-hCD20 ublituximab directly into the tumor bed. Mice then received an intraperitoneal injection of luciferin every 3 to 5 days and the luminescence was analyzed with the IVIS-lumina II device. Figure 3 are the representative results from 1 out of the 7 mice. The scale on the right of the photographs represents luminescence intensity, red being more intense.

Dose-Response After Intrathecal Injection

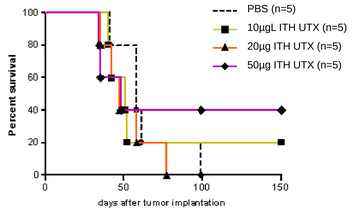


Figure 4: Kaplan-Meier survival analysis of mice inoculated intracerebrally with A20.IIA-GFP-hCD20 tumor cells and treated 7 days later with a single injection of PBS, 10µg, 20µg or 50µg of anti-hCD20 ublituximab in the *cisterna magna*. Figure 4 is one representative out of three experiments conducted.

Ublituximab vs. Rituximab

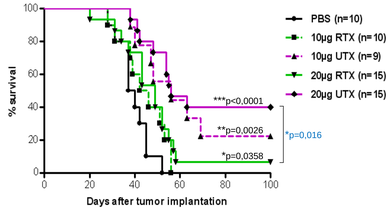
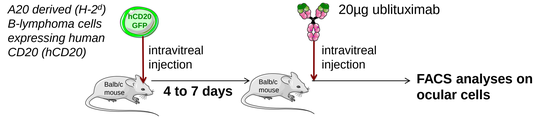


Figure 5: Comparison of the therapeutic efficiency of ublituximab versus rituximab in the PCL model. Mice were implanted with hCD20 expressing tumor cells and one week later received 10 or 20µg of UTX or RTX as a treatment. Their survival was then followed and a Kaplan-Meier analysis was performed with the Log-rank test between the control and the treated groups (*) as well as between the 20µg UTX treated group and the 20µg RTX treated group, as indicated (*)

Anti-Tumor Effect of Ublituximab in a Primary Intraocular Lymphoma (PIOL) Model

Experimental Setting



Additional information on the PIOL model can be found in reference [3]

Kinetic Effect of Ublituximab

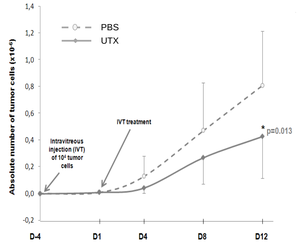


Figure 6: Flow cytometric analysis of the number of CD19+ GFP+ tumor cells among total ocular living cells at 5, 8, 12 and 16 days after tumor implantation. Animals were treated with a single injection of either PBS or 20µg of UTX 4 days after the tumor implantation, as indicated on the graph.

Mann & Whitney statistical test.

Tumor Burden Inhibition by Ublituximab

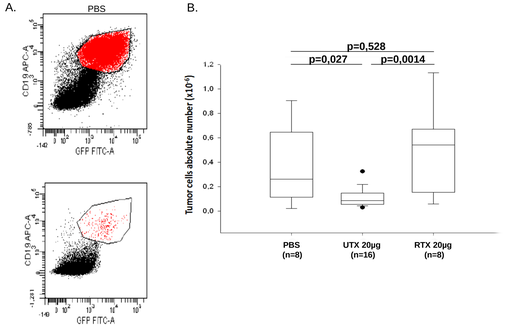


Figure 7: Flow cytometric analyses showing the inhibition of tumor growth after UTX injection. Mice received the tumor intravitreally at Day 0 and were treated once with either 20µg ublituximab or 20µg rituximab at Day 7. Eyes were then harvested and analyzed for the presence of CD19+ GFP+ cells. **(A)** Representative dot plots showing CD19+ GFP+ cells in a non treated (top panel) or UTX treated (bottom panel) eye. **(B)** Box plot analysis of the absolute number of tumor cells in non treated (left), UTX treated (middle) or RTX treated (right) mice. *Mann & Whitney statistical test.*

CONCLUSIONS

- **Ublituximab exhibited a marked anti-tumor effect from a single dose, especially in the PCL model where some animals experienced complete elimination of tumor**
- **Ublituximab treatment decreased the number of tumor cells and concomitantly increased CD8+ T-cells following treatment, suggesting the implication of the adaptive arm of the immune system**
- **Optimal efficacy of ublituximab was achieved with 20µg and was superior to rituximab at the same dose**
- ***In vivo* results confirm the potential of the glycoengineered anti-hCD20 ublituximab as an innovative therapeutic approach for the treatment of PCNSL tumors, as well as other B-cell lymphomas**
- **Future clinical trials in primary CNS lymphomas are warranted**

[1] De Romeuf *et al.*, 2008, Br.J.Haematol., 140(6):635, [2] Donnou *et al.*, 2011, Clin. Exp. Immunol., 165(3):329, [3] Touitou *et al.*, 2007, IOVS, 48(7):3223