PS1310

The novel bispecific CD47-CD19 antibody TG-1801 potentiates the activity of Ublituximab-Umbralisib (U2) drug combination in preclinical models of B-cell non-Hodgkin lymphoma

Marcelo L Ribeiro[1], Emmanuel Normant[2], Diana Reyes Garau[1], Hari P. Miskin[2], Peter Sportelli[2], Michael S. Weiss[2], Francesc Bosch[1] and Gael Roué[1]

[1]Department of Hematology, University Hospital Vall d'Hebron, Vall d'Hebron Institute of Oncology (VHIO), Autonomous University of Barcelona, Barcelona, Spain; [2] TG Therapeutics, New York, NY, USA.

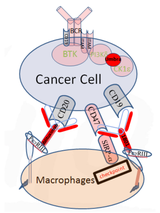
BACKGROUND:

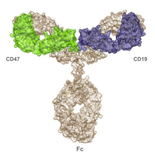
Deregulated BCR signaling is considered to be a key contributor to tumor growth and survival in B-cell non-Hodgkin lymphoma (B-NHL). Targeting this pathway led to the development of inhibitors of Bruton's tyrosine kinase (BTK) and phosphatidylinositol 3 kinase (PI3K), which are validated therapeutic strategies in B-NHL.[1,2]

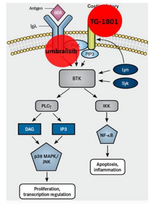
Recently, it has been reported that targeting CD47, a dominant "don't eat me" signal for macrophages, represents a novel therapeutic strategy for enhancing antitumor responses mediated by the innate immune system.[3]

Herein we studied the effects of TG-1801, a novel CD47-CD19 bispecific antibody, *in-vitro* and *in-vivo*, on the antitumor activity of ublituximab, umbralisib, and the combination of both ("U2").

- **Ublituximab** : Next-generation glycoengineered anti-CD20 monoclonal antibody, in Phase 3 pivotal trials.[4]
- **Umbralisib**: PI3Kδ and CK1ε dual inhibitor which has demonstrated activity in preclinical models and primary B-NHL cells, and in patients with B-cell malignancies. The doublet of umbralisib and ublituximab (called the "U2" regimen), provides a non-chemotherapy backbone regimen on which novel multidrug combinations have been explored.[5]
- **TG-1801** : CD47-CD19 bispecific antibody, currently in Phase 1, that selectively targets CD47 on CD19+ B-cells, sparing red blood cells and platelets, and blocking the CD47-SIRPα macrophage checkpoint on mature B cells.



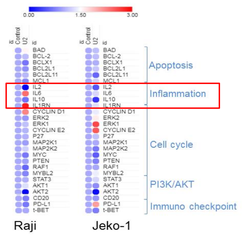


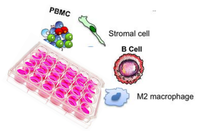


AIM:

To evaluate the synergistic anti-tumor activity of the novel bispecific CD47-CD19 antibody TG-1801 in combination with the anti-CD20 mAb ublituximab and the PI3Kδ-CK1ε dual inhibitor umbralisib in *in vivo* and *in vitro* models of B-NHL.

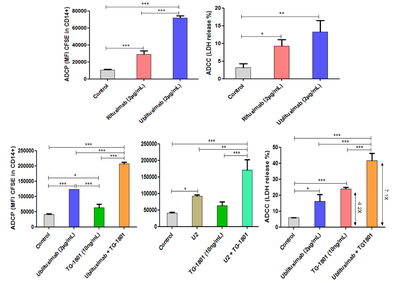
RESULTS 1: *In vitro co-culture assays*



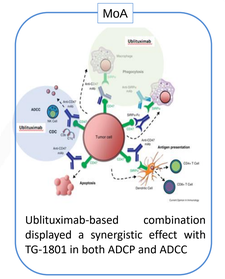


- Umbralisib + ublituximab "U2" mainly regulates the expression of inflammation-related genes in B-NHL co-cultures

Fig. 1. Effect of U2 on B-cell signaling pathways. Raji (Burkitt lymphoma) and Jeko-1 (mantle cell lymphoma) cell lines were co-cultured with bone marrow-derived stromal cells, M2-polarized primary macrophages, and PBMCs (4:1:1:1) and treated with U2 combination (umbralisib 1μM + ublituximab 2μg/mL) for 24h. B-cells were isolated using EasySep™ Human B Cell Enrichment Kit (StemCell Technologies) before RNA extraction. qPCR was performed using a set of B-NHL related genes. Data are presented in fold-change relative to control.



- Ublituximab exhibited a stronger CD20-mediated ADCP and ADCC compared to rituximab.
- Ublituximab and U2 lead to a 2-fold increase in ADCP, which is doubled to 4-fold in combination with TG-1801.
- TG-1801 single agent leads to an increase of 4.2-fold above control in ADCC. This value reaches 7.1-fold when combined with ublituximab.



Ublituximab-based combination displayed a synergistic effect with TG-1801 in both ADCP and ADCC

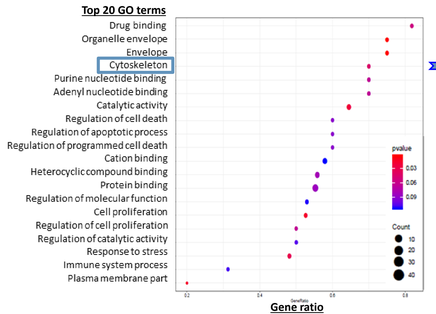
Fig. 2. Opsonizing ublituximab increases TG-1801-driven ADCC and ADCP. **Antibody-dependent cell phagocytosis (ADCP)** was assessed by pre-treating the CFSE-labelled Raji cells with the indicated antibodies for 30 min before their incubation with M2 macrophages (ratio 1:4) for 1 hour. Shown are the percentages of B-cells-containing macrophages (CD14+/CFSE+) as detected by flow cytometry. **Antibody-dependent cellular cytotoxicity (ADCC)** was assessed by pre-treating Raji cells with antibodies or isotype control for 30 min. PBMCs (E:T 10:1) were added to the target cells and co-cultured for 4 h. LDH release from target cells was quantified using Cytotoxicity Detection Kit^{PLUS} (Sigma Aldrich).

Treatment	GO	*NES	p value
Control vs U2	Oxidation-reduction process	-0.56	0.016
U2 vs 1801 + U2	Organelle envelope	-0.56	0.006
	Envelope	-0.56	0.006
	Plasma membrane part	-0.55	0.016
	Cell proliferation	-0.43	0.017
	Catalytic activity	-0.35	0.023
	Response to stress	0.32	0.032
	Cytoskeleton	-0.51	0.046

*Normalized enrichment score

- RNA-seq analysis reveals that U2 treatment is mainly associated with a reduction of cellular respiration (redox processes) in B-NHL co-cultures, consistent with the role of PI3K in the control of malignant B cell metabolism.[6]
- The combination TG-1801+U2 leads to a down-regulation of genes associated with cell architecture homeostasis, including cellular membranes, cytoskeleton and cell proliferation.

Fig. 3. Raji and Jeko-1 cells were co-cultured with bone marrow-derived stromal cells, M2-polarized primary macrophages and PBMCs (4:1:1:1) and treated with U2 combination (umbralisib 1μM + ublituximab 2μg/mL), TG-1801 (10 ng/mL) or the TG-1801 + U2 combination for 24h. B-cells were isolated using EasySep™ Human B Cell Enrichment Kit (StemCell Technologies) for RNA extraction. RNA-seq was performed at CNAG (Barcelona) and GSEA analysis at CBMSO (Madrid).



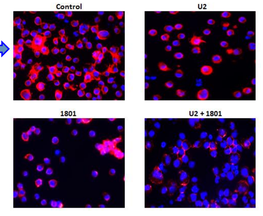
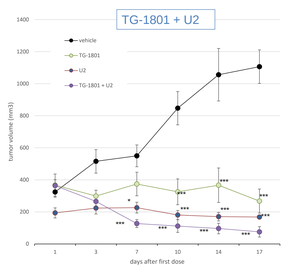
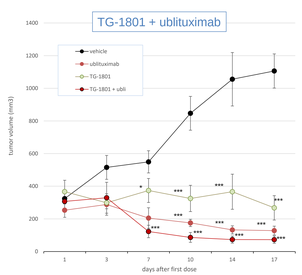


Fig. 4. U2 and TG-1801 cooperate to decrease F-actin polymerization. Raji cells were treated with U2, TG-1801, or TG-1801 + U2 combination for 24h. F-actin polymerization was visualized by labelling the cells with phalloidin-TRITC (red). Nuclei were stained by using DAPI (blue).

- U2 and TG-1801 cooperate by promoting F-actin disruption, a hallmark of responsiveness to anti-CD47 therapy in malignant B cells.[7]

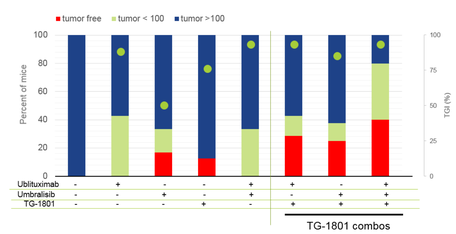
RESULTS 2: *Raji xenograft model*





TG-1801 cooperates with ublituximab (right panel) and with the ublituximab + umbralisib combination (U2, left panel) to reduce tumor growth in a subcutaneous mouse model of Burkitt lymphoma. NSG mice were subcutaneously injected with Raji cells and tumor-bearing mice were randomly assigned to one of the following treatment arms (8-6 mice per group): Ublituximab (5mg/kg, qw), Umbralisib (150mg/kg, bid), TG-1801 (5mg/kg, qw), the combinations of U2, TG-1801 + Ublituximab or the triple combo (U2 + TG-1801) for 17 days.

- When administrated alone, ublituximab, ublituximab + umbralisib (U2) and TG-1801 displayed a tumor growth inhibition (TGI) of 88%, 85% and 76%, respectively.
- The combinations of TG-1801 with ublituximab and U2 achieved TGI of 93% and 93% respectively.



TG-1801 cooperates with ublituximab and with the ublituximab + umbralisib (U2) combination to reduce tumor growth in a subcutaneous mouse model of Burkitt lymphoma. The anti-tumor activity of ublituximab and TG-1801 was too strong to clearly delineate an additive effect at the end of treatment (d17, left figures). The treatments were then stopped and mice with no tumor or low tumor size were kept alive for another 35 days. The green dot represent the TGI%, aligned on the right side Y axis.

- Thirty five days after the last dose (d42) all the mice either tumor-free (red) or bearing a small tumor (green, <100mm3) were alive. The TG-1801 combo groups showed an increased number of tumor free or low tumor burden bearing mice (red and green bars).

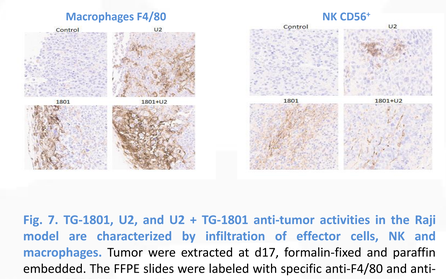


Fig. 7. TG-1801, U2, and U2 + TG-1801 anti-tumor activities in the Raji model are characterized by infiltration of effector cells, NK and macrophages. Tumor were extracted at d17, formalin-fixed and paraffin embedded. The FFPE slides were labeled with specific anti-F4/80 and anti-mouse CD56 (Histotox, CO)

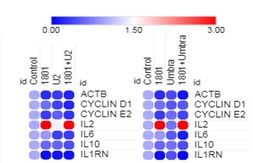


Fig. 8. Effect of TG-1801, umbralisib, U2 and combinations on gene expression. Tumor were extracted at d17, and qPCR was performed. Data are presented in fold-change relative to vehicle group (control).

- Umbralisib and U2 cooperate with TG-1801 to decrease β-actin (ACTB) expression
- Umbralisib and U2 cooperate with TG-1801 to downregulate cell cycle controlling genes
- TG-1801 treatment showed a strong pro-inflammatory effect which was enhanced by the tested combinations.

Conclusions:

- Ublituximab exerts stronger ADCP and ADCC activity in B-NHL cells when compared to rituximab
- TG-1801 increased the ADCC and ADCP activities initiated by both ublituximab and the U2 combination.
- TG-1801 + U2 regulates genes related with cell architecture.
- TG-1801 triggers synergistic tumor growth inhibition and results in prolonged remission when added to U2 in the *in vivo* Raji lymphoma model. This phenomenon may be mediated by increased infiltration of immune effector cells.
- A dose-escalation Phase 1 clinical trial of TG-1801 is ongoing in histologically confirmed B-cell lymphoma relapsed or refractory to prior standard therapy.

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