Poster # 269

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

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Abstract

Umbralisib is an orally available PI3Kδ inhibitor that has demonstrated activity in preclinical models and primary B-NHL cells, and in patients with B-cell malignancies. The doublet of umbralisib used in combination with the glycoengineered anti-CD20 mAb ublituximab ("U2" regimen), provides a non-chemotherapy backbone regimen on which novel multidrug combinations have been explored. TG-1801 is a novel, bispecific antibody currently in Phase 1 that selectively targets CD47 on CD19+ B-cells, sparing red blood cells or platelets, and blocking the CD47-SIRPα macrophage checkpoint on mature B cells. Here we show that activity of the U2 combination in B-NHL cell lines co-cultured with bone marrow-derived stromal cells, M2-polarized primary macrophages, and primary circulating PBMCs is mainly associated with a decrease of anti-inflammatory cytokines genes (IL-6, IL-10, IL-1RA). In addition, upregulation of pro-inflammatory IL-2, accompanied by a 2-fold increase of M2-dependent antibody-dependent cell phagocytosis (ADCP) is also observed. Notably, when U2 is combined with TG-1801, this 2-fold increase in ADCP doubled up to 4-fold increase. Moreover, the activated antibody-dependent cell death (ADCC) observed with TG-1801 alone (4.1-fold above control) became increased (7.2-fold above control) when combined with ublituximab, whereas umbralisib alone did not affect ADCC. RNA-seq analysis further reveals that U2 treatment is mainly associated with modulation of redox processes in B-NHL co-cultures (NES=-0.57, p=0.016). Although TG-1801 single agent does not significantly affect B cell transcriptome, combination with U2 provokes the down-regulation of genes associated to cell architecture homeostasis, including cellular membranes (NES=-0.57, p=0.006), cytoskeleton (NES=-0.51, p=0.046), and cell proliferation (NES=-0.44, p=0.018). *In vivo*, treatment with ublituximab alone (5mg/kg, qw) displays a tumor growth inhibition (TGI) of 88%, with 3/8 mice harboring a barely palpable tumor, while treatment with umbralisib alone (150mg/kg, bid) shows a TGI of 50%, with 2/8 mice lacking detectable tumors. TG-1801 (5mg/kg, qw) exhibits a 76% TGI with 1/8 tumor free-mouse. Most importantly, the combination of TG-1801 with umbralisib alone, ublituximab alone, and U2 achieves TGI of 85%, 93% and 93% respectively, and 35 days after the last dose 3/8 mice remain tumor-free in the triple combo groups while only the case for 1/8 mouse in the TG-1801 group. Interestingly, this superior anti-tumor effect of the TG-1801-based combinations is associated with a higher infiltration of mouse macrophages within the tumors, as assessed by F4/80 IHC labeling. In conclusion, the results presented herein set the preclinical rationale for a combination strategy of the novel CD47-CD19 antibody TG-1801 with other B-cell targeted drugs, particularly umbralisib and ublituximab, in B-NHL.

AIM:

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.

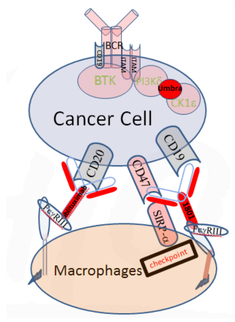
BACKGROUND:

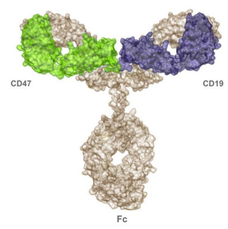
Deregulated BCR signaling is considered to be a key contributor to tumor growth and survival in B-cell non-Hodgkin lymphoma (B-NHL). Targeting this pathway led to the development of inhibitors of Bruton's tyrosine kinase (BTK) and phosphatidylinositol 3 kinase (PI3K), which are validated therapeutic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

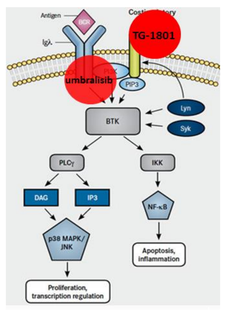
Recently, it has been reported that targeting CD47, a dominant "don't eat me" signal for macrophages, represents a novel therapeutic strategy for enhancing antitumor responses mediated by the innate immune system.[3]

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.



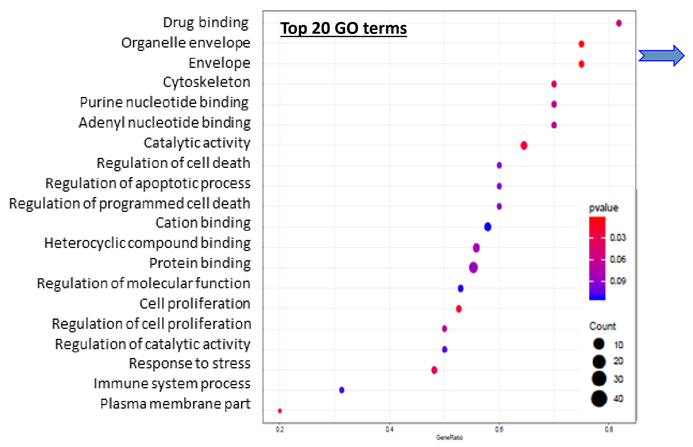




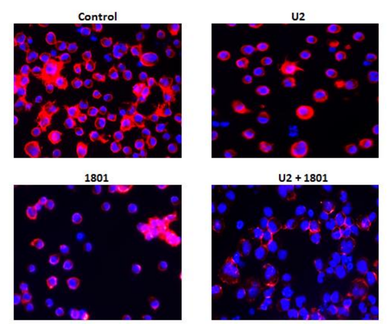
In vitro co-culture

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

- 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.



Gene ratio

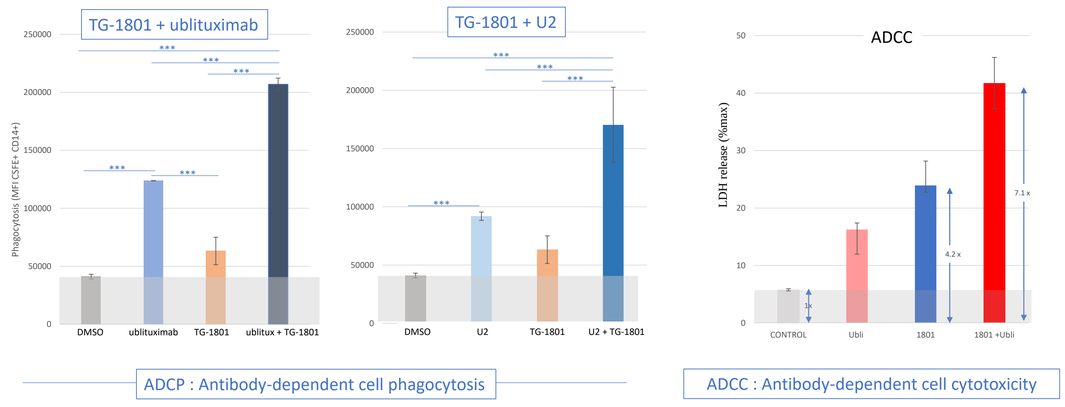


FU2 and TG-1801 cooperate to decrease F-actin polymerization. Raji cells were treated with U2, TG-1801 or TG-1801 + U2 combination for 24h. F-actin polymerization was visualized by labelling the cells with phalloidin-TRITC (red). Nuclei were stained by using DAPI (blue).

. 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).

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

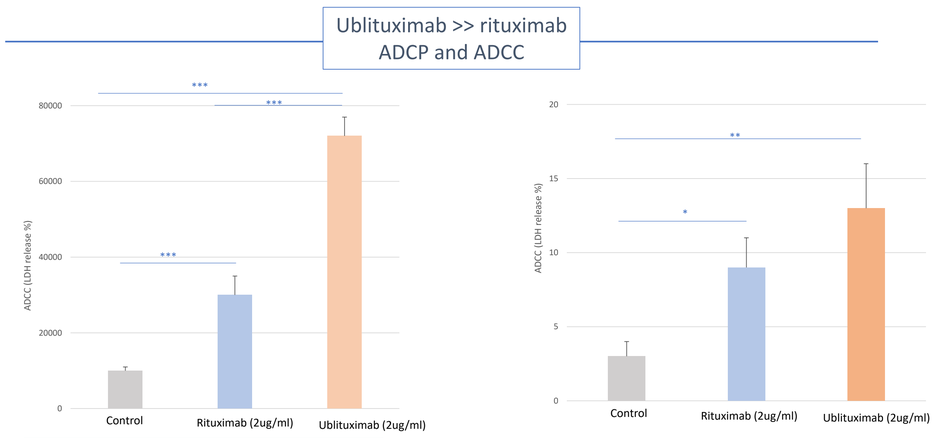
In vitro ADCC and ADCP



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 PBMC-derived macrophages (ratio 1:4) for 1 hour. Shown are the percentages of B-cells-containing macrophages (CD14+/CFSE+) as detected by flow cytometry.

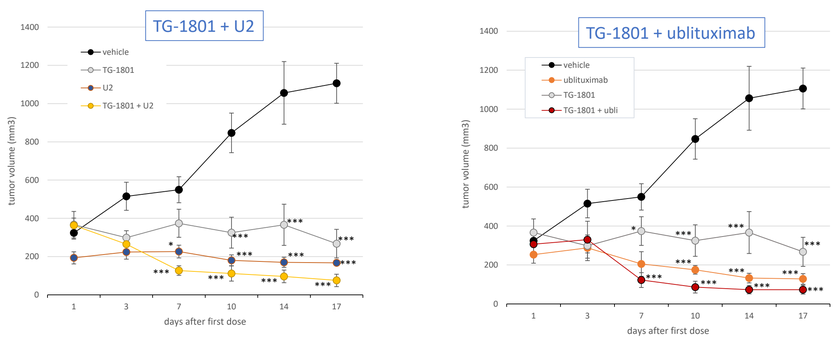
Antibody-dependent cellular cytotoxicity (ADCC) was assessed by pre-treating Raji cells with antibodies or isotype control for 30 min. PBMCs (E:T 10:1) were added to the target cells and co-cultured for 4 h. LDH release from target cells was quantified using Cytotoxicity Detection Kit[PLUS] (Sigma Aldrich).



Opsonizing Ublituximab is more potent than rituximab to trigger ADCP and ADCC

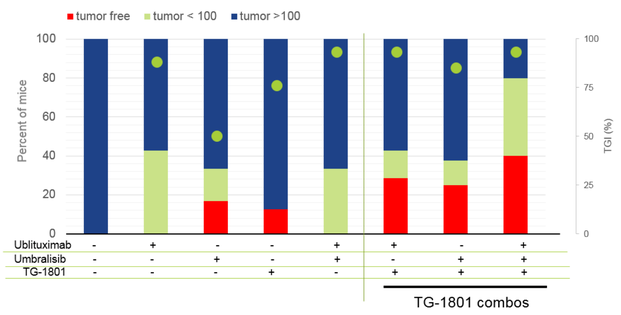
ADCP was assessed by pre-treating the CFSE-labelled Raji cells with the indicated antibodies for 30 min before their incubation with M2 macrophages (ratio 1:4) for 1 hour. Percentages of B-cells-containing macrophages (CD14+/CFSE+) as detected by flow cytometry are reported. ADCC was assessed by pre-treating 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).

In vivo, 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.

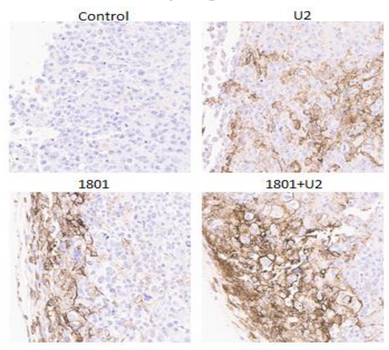


TG-1801 cooperates with ublituximab and with the ublituximab + umbralisib (U2) combination to reduce tumor growth in a subcutaneous mouse model of Burkitt lymphoma. The anti-tumor activity of ublituximab and TG-1801 was too strong to clearly delineate an additive effect at the end of treatment (d17, 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.

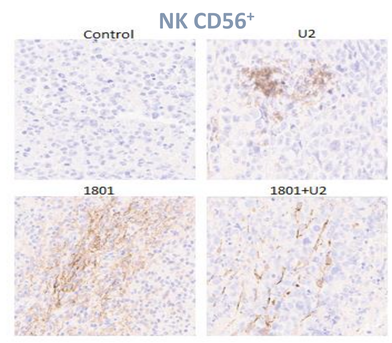
- Thirty five days after the last dose (d42) all the mice either tumor-free (red) or bearing a small tumor (green, <100mm3) were alive. The TG-1801 combo groups showed an increased number of tumor free or low tumor burden bearing mice (red and green bars).

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)

Macrophages F4/80



NK CD56+



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.

References:

1. Niemann CU, Wiestner A. B-cell receptor signaling as a driver of lymphoma development and evolution. Semin Cancer Biol. 2013.
2. Mahadevan D, Fisher RI. Novel therapeutics for aggressive non-Hodgkin's lymphoma. J Clin Oncol. 2011.
3. Chao MP, Tang C, Pachynski RK. Et al. Extranodal dissemination of non-Hodgkin lymphoma requires CD47 and is inhibited by anti-CD47 antibody therapy. Blood. 2011.
4. Babiker HM, Glode AE, Cooke LS, Mahadevan D. Ublituximab for the treatment of CD20 positive B-cell malignancies. Expert Opin Investig Drugs. 2018.
5. Burris HA 3rd, Flinn IW, Patel M. et al. Umbralisib, a novel PI3Kδ and casein kinase-1ε inhibitor, in relapsed or refractory chronic lymphocytic leukaemia and lymphoma: an open-label, phase 1, dose-escalation, first-in-human study. Lancet Oncol. 2018.
6. Vangapandu HV, Havranek O, Ayres ML, et al. B-cell Receptor Signaling Regulates Metabolism in Chronic Lymphocytic Leukemia. Mol Cancer Res. 2017.
7. Barbier S, Chatre L, Bras M. et al. Caspase-independent type III programmed cell death in chronic lymphocytic leukemia: the key role of the F-actin cytoskeleton. Haematologica. 2009.

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