Antitumoral activity of the novel BTK inhibitor TG-1701 is associated with disruption of Ikaros signaling and improvement of anti-CD20 therapy in B-cell non-Hodgkin lymphoma

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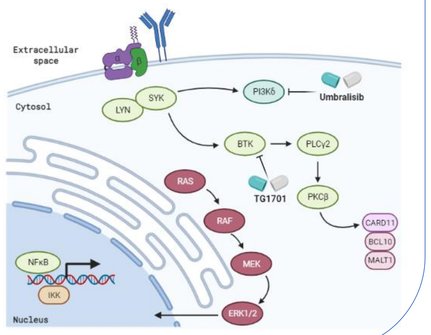
Background

Introduction: B-cell non-Hodgkin lymphomas (B-NHLs) account for up to 4% of globally diagnosed cancers. Targeting of the B-cell receptor (BCR) pathway through inhibition of Bruton's tyrosine kinase (BTK) with the first-in-class irreversible inhibitor ibrutinib has demonstrated exceptional clinical activity as a monotherapy for various subtypes of B-NHL. However, its activity has been limited by 1) acquired resistance due to the development of a cysteine to serine mutation at the BTK catalytic site (BTK^{C481S}) or over-activation of the NF-kB pathway, and 2) off-target activity that introduces toxicity and also precludes their use in combination with anti-CD20 antibodies.



- TG-1701 is a novel irreversible and highly specific BTKi currently under study in patients with relapsed/refractory (R/R) B-NHL alone and in combination with ublituximab, a novel glycoengineered anti-CD20 antibody, and umbralisib, a dual PI3Kδ and casein kinase-1ε inhibitor (combination also referred to as U2 regimen).

TG-1701 is a novel clinical irreversible BTKi

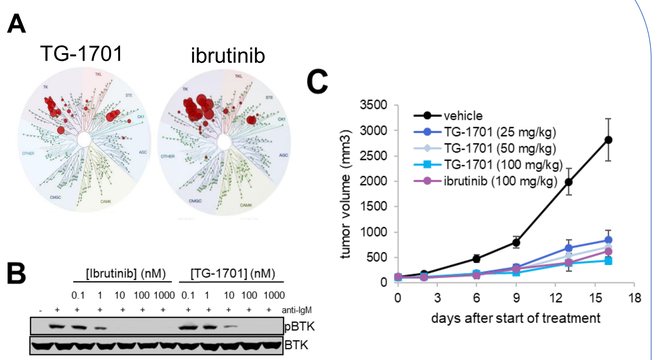


Fig. 1 - (A) Binding of TG-1701 and ibrutinib (1 µM) was tested in a panel of 441 kinases using the DiscoverX technology. TG-1701 showed a comparable BTK Kd (3 nM vs 1.5 nM, respectively) and a lower binding to EGFR, ITK, TXK, and JAK3 (Kd 135-, >48-, 68- and >94-fold higher than those of ibrutinib, respectively. The size of each red circle is proportional to the strength of the binding. (B) DoHH-2 cells were incubated with increasing doses of ibrutinib or TG-1701, followed by BCR stimulation with 10 µg/mL goat F(ab')2 anti-IgM for 18h. Phospho-Btk (Tyr223) levels were assessed by western blotting. (C) TG-1701 or ibrutinib were dosed orally in the Mino MCL xenograft model. The tumor growth inhibition (TGI) achieved by a 16-day treatment with 25, 50 and 100 mg/kg TG-1701 (56%, 72% and 78%, respectively), was comparable to the 70% TGI observed in the ibrutinib (100 mg/kg) arm.

Phosphoproteomics may differentiate early and late CLL responders to TG-1701

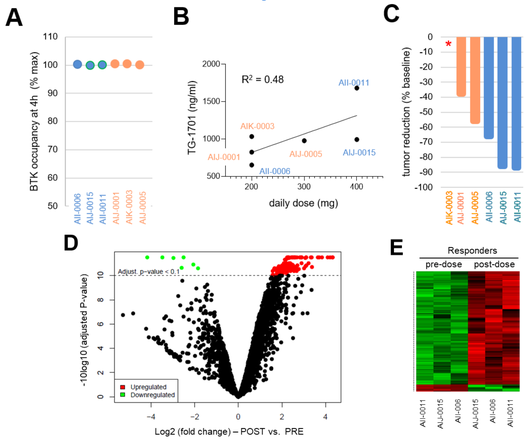


Fig. 2 Pre- and 4h post-dose samples from six CLL patients treated with TG-1701 were analyzed using phosphoproteomics. Unsupervised clustering of the data selected to uncover changes due to TG-1701 treatment clearly fitted the clinical outcome of the patients, i.e. early (blue) and late-responders (orange). (A) BTK occupancy was assessed in the 6 studied CLL patient samples from the Phase 1 dose-escalation study (NCT03671590). Figure 2A shows that the differentiation between the 2 groups is not related to the level of BTK occupancy. (B) PK: the dose-response in the 6 patients also showed that the grouping is not related to exposure. (C) Best tumor reduction in all six patients at C3D1. *AIK-003 lymphocytosis at C3D1 ranked the response as a stable disease. (D) Volcano plot of the quantified phosphopeptides in early responders only. The late responders did not exhibit any TG-1701-driven changes. (E) The phosphosites up (red) or down (green) regulated in figure D are shown for each three single early responder patients.

Impairment of Ikaros signaling is associated with clinical response to TG-1701

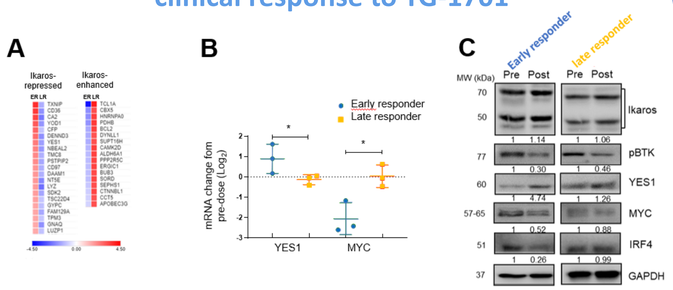


Fig. 3 - Regulation of Ikaros-regulated factors upon TG-1701 treatment in early responders (ER) and late responders (LR), according to (A) total proteome analysis, (B) qPCR detection of *YES1* (an Ikaros-repressed gene) and *MYC* (an Ikaros-enhanced gene) and (C) Immunoblot evaluation of p-BTK, Ikaros and Ikaros downstream genes, using representative early responder and late responder PBMC lysates (values below each lane represent the quantification related to GADPH).

TG-1701 treatment leads to BTK-dependent disruption of Ikaros transcriptional program

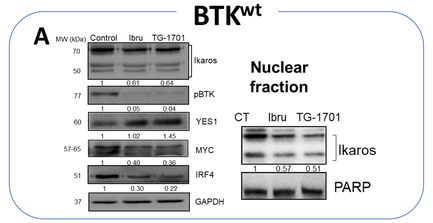


Fig. 4 (A) - MCL REC-1 cells were treated for 24h with 1 µM ibrutinib or TG-1701, and variations in Ikaros-regulated factors were quantified using western blot (left panel). Subcellular localization of Ikaros was determined by immunoblot analysis of nuclear protein fraction (right panel).

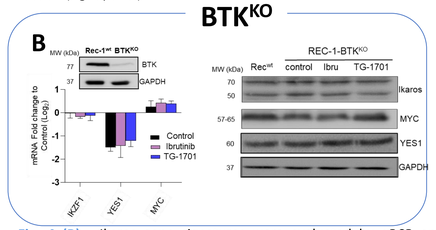


Fig. 4 (B) - Ikaros gene signatures were evaluated by qPCR and western blot in REC-1 and in CRISPR-Cas9-engineered REC-1-BTK^{KO} cells exposed for 24h to 1 µM ibrutinib or TG-1701.

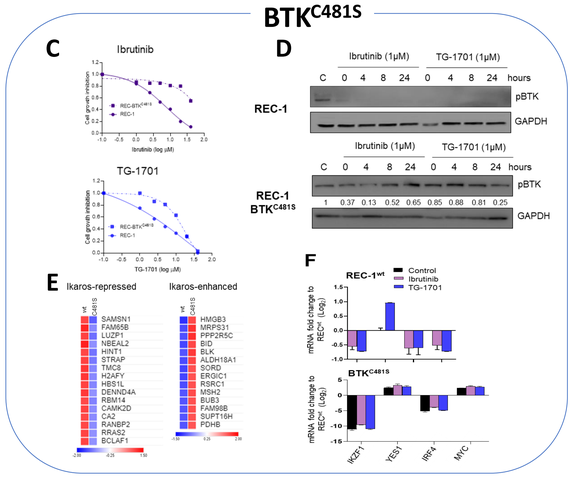


Fig. 4 - (C) Viability of BTK^{wt} and CRISPR-Cas9-engineered BTK^{C481S} REC-1 cells exposed to increasing doses of ibrutinib or TG-1701. (D) Cells were exposed for 1h to 1µM TG-1701, washed-out for the indicated times, and levels of phospho- and total BTK were assessed using immunoblotting. (E) Regulation of Ikaros-regulated factors after 4 h of treatment with TG-1701 or ibrutinib (1 µM) in REC-1 and REC-1-BTK^{C481S} cells according to total proteome data. (F) Ikaros gene signatures were evaluated by qPCR in cells exposed for 24 h to 1 µM ibrutinib or TG-1701.

TG-1701 does not impair FcγR-driven ADCC and ADCP and cooperates with U2 in BTKi-sensitive and BTKi-resistant models of B-NHL

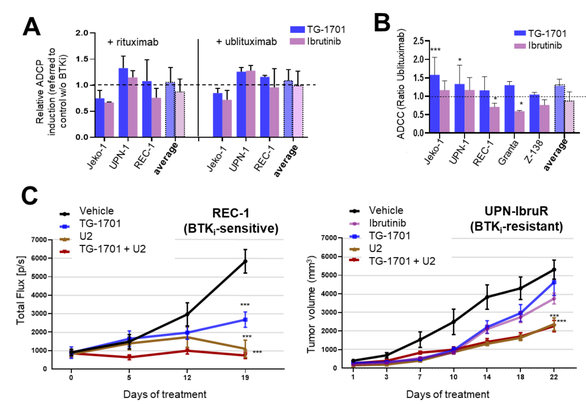


Fig. 5 - Sustained CD20 antibody-driven ADCC and ADCP upon TG-1701 in *in vitro* and *in vivo* models of MCL. (A) Engulfment of CFSE-labelled MCL cell by primary M1-polarized macrophages was evaluated following treatment with 2 µg/mL anti-CD20 ± 1 µM BTKi. The data show the relative antibody-dependent cell phagocytosis (ADCP) induction (referred to control w/o BTKi) in B-cells-containing CD14+/CFSE+ macrophages, as detected by flow cytometry. (B) Antibody-dependent cellular cytotoxicity (ADCC) was assessed in a panel of MCL lines pre-treated with ublituximab (2 µg/mL) in combination with 1 µM of ibrutinib or TG-1701, and incubated with fresh PBMCs. ADCC induction was quantified by LDH release assay and referred to ublituximab as single agent. (C) TG-1701, ibrutinib (in UPN-IbruR), U2 regimen and TG-1701+U2 combinations were dosed orally in BKTi-sensitive (REC-1-GFP+LUC+) and NF-κB-driven, BTKi-resistant (UPN-IbruR)[1] MCL xenograft models. *p < 0.05, **p < 0.01, ***p < 0.001, and ns = non-significant.

[1]Balsas P, et al. *J Hematol Oncol* 2017;10:80.

Conclusions

- TG-1701 is a novel irreversible BTK inhibitor currently in Phase 1 clinical development, as monotherapy or in combination with ublituximab and umbralisib.
- In patient samples from a Phase 1 clinical trial of TG-1701, phosphoproteomic analysis differentiated early and late CLL responders to TG-1701 therapy.
- Disruption of an active Ikaros pathway is a signature of early responders, while absence of Ikaros modulation upon TG-1701 therapy is a signature of non-/late responders.
- TG-1701 did not impair FcγR-driven ADCC and ADCP and cooperated with U2 in *in vitro* and *in vivo* models of BTKi-sensitive and BTKi-resistant B-NHL.

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