TG-1701, a novel irreversible Bruton's kinase (BTK) inhibitor, cooperates with ublituximab-driven ADCC and ADCP in *in vitro* and *in vivo* models of ibrutinib-resistant mantle cell lymphoma

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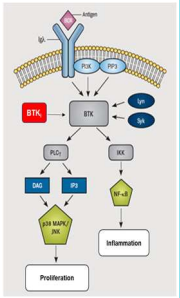
BACKGROUND:

- Mantle cell lymphoma is a rare but challenging subtype of B-cell non-Hdgkin lymphoma that generally responds to initial treatment but inevitably relapses, making it incurable with standard chemotherapy. The clinical presentation of MCL varies widely. Some patients have an indolent disease course with longer survival, and others can have a very aggressive course with shorter survival[1].
- The first-in-class Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib, has proven to be an effective agent for patients with relapsed/refractory MCL. The development of a cysteine to serine mutation at the BTK catalytic site (BTK^{C481S}) or over-activation of the NF-kB pathway can impair MCL response to most BTK inhibitors (BTKis)[2].
- TG-1701 is a novel irreversible inhibitor highly specific to BTK, with improved selectivity when compared to ibrutinib, currently being evaluated in a phase 1 clinical trial in NHL and chronic lymphocytic leukemia (CLL) patients, alone or in combination with the anti-CD20 mAb ublituximab and the PI3Kδ/CK1ε inhibitor umbralisib ("U2 regimen").[3]
- TG-1701 is as active as ibrutinib but with improved selectivity[3].



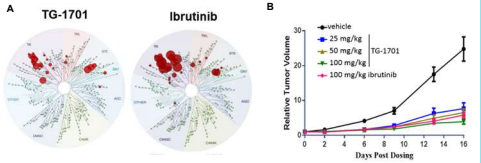


Fig. 1. (A) Binding of TG-1701 and ibrutinib 1 uM was tested in a panel of 441 kinases using the Discoverex kinomeSCAN technology. The size of each red circle is proportional to the strength of the binding. (B) Nude mice were subcutaneously injected with DOHH-2, an NHL cell line, and tumor-bearing mice were randomly assigned to one of the following treatment arms : TG-1701 (25, 50, 100 mg/kg, PO, bid) and ibrutinib (100mg/kg, PO, bid), for 21 days. Panel C shows a similar anti-tumor effect of ibrutinib and TG-1701 in this model.

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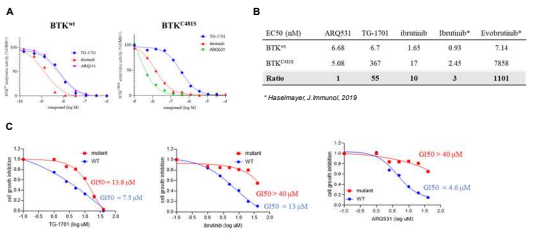
RESULTS #1: TG-1701 retains some activity in ibrutinib-resistant BTK^{C481S} MCL cells



B	EC50 (nM)	ARQ531	TG-1701	ibrutinib	Ibrutinib*	Evobrutinib*
	BTK^{wt}	6.68	6.7	1.65	0.93	7.14
	BTK^{C481S}	5.08	367	17	2.45	7858
	Ratio	1	55	10	3	1101

* Haselmayer, J Immunol, 2019

Fig. 2 (A-B) Ibrutinib, ARQ531 (reversible BTK inhibitor) and TG-1701, were tested in a BTK^{wt} and BTK^{C481S} radioactive [33P] ATP enzymatic filtration assay. In this assay, all BTK^{wt} EC50s reported were in agreement with published data. As expected, the reversible inhibitor ARQ-531 showed similar potency toward the wild type and the mutant BTK (6.68 and 5.08 nM, respectively). Interestingly, ibrutinib showed a 10-fold decrease in potency (1.65 nM and 17 nM, respectively), in the range of the 3-fold reported previously[4], whereas more selective irreversible BTK inhibitors, TG-1701 and evobrutinib were 55- and 110-fold, respectively, less potent against BTK^{C481S}, suggesting a correlation between higher selectivity and dependency to Cys 481. (C) Using a CRISPR-engineered MCL cell line, REC-1 BTK^{C481S}, cell viability was evaluated using a 72h CellTiter-Glo luminescent assay (Promega). REC-1 cells harbor a mild dependency on BTK for their growth. In this assay, only TG-1701 was showing some inhibitory activity against REC-1 BTK^{C481S} growth.

RESULTS #2: NF-κB-driven ibrutinib refractoriness impairs TG-1701 activity *in vitro* and *in vivo* in MCL

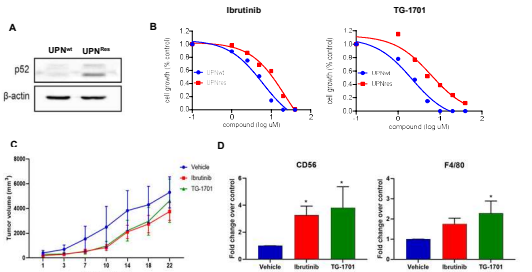


Fig. 3 (A) The UPN1res MCL cell line was derived from the parental UPN1 by repeated drug selection. UPN1^{Res} resistance to ibrutinib was linked to the activation of the non-canonical NF-κB pathway[5]. (B) Cell viability was performed using a CellTiter-Glo luminescent assay (Promega) using increasing concentrations of TG-1701 or ibrutinib for 72h. When compared with the parental cell line, UPN1res showed a consistent 3-fold shift in IC50, both with ibrutinib and TG-1701. (C) NSG mice were subcutaneously injected with UPN1res cells, and tumor-bearing mice received vehicle, 25 mg/kg daily ibrutinib or TG-1701 by oral gavage. Both ibrutinib and TG-1701 showed a modest antitumor effect (TGI 29% and 17%, respectively), in agreement with the modest *in vitro* activity. (D) RNA was isolated and the expression of the NK cell marker CD56 in ibrutinib and TG-1701 treated groups was ~3.5-fold higher than in control group (p=0.0487 and 0.0169, respectively). In addition, ibrutinib lead to a slight non-significative increase in F4/80 levels, a mouse macrophage marker (1.75-fold, p=0.14), whereas TG-1701 induced a 2.3-fold increase (p=0.0139).

RESULTS #3: Ibrutinib, but not TG-1701, blocked ADCC and ADCP triggered by the anti-CD20 antibody ublituximab

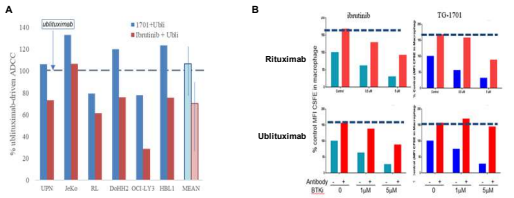


Fig. 4 (A) Antibody-dependent cellular cytotoxicity (ADCC) was assessed by pre-treating cells with antibodies or isotype control for 30 min. PBMCs (E:T 10:1) were added to the target cells and co-cultured for 4 h. LDH release from target cells was quantified using Cytotoxicity Detection Kit^{PLUS} (Sigma Aldrich). In six different cell lines, ibrutinib clearly inhibited ADCC, whereas TG-1701 did not. (B) Antibody-dependent cell phagocytosis (ADCP) was assessed by pre-treating the CFSE-labelled cells with the indicated antibodies for 30 min before their incubation with M2 macrophages (ratio 1:4) for 1 hour. The data show the percentages of B-cells-containing macrophages (CD14+/CFSE+) as detected by flow cytometry. The dotted line represents ADCP activity with each antibody alone. Our data show that TG-1701, in contrast to ibrutinib, did not exhibit any negative effect on ublituximab-derived phagocytosis

RESULTS #4: *In vivo* TG-1701 demonstrates additive anti-tumor inhibition when combined with ublituximab and umbralisib (U2) regimen

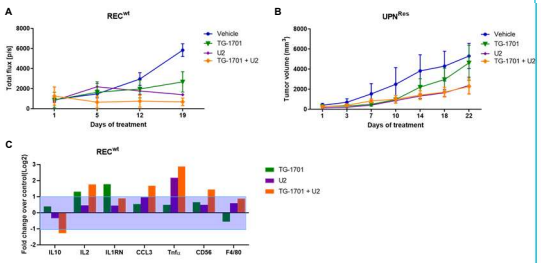
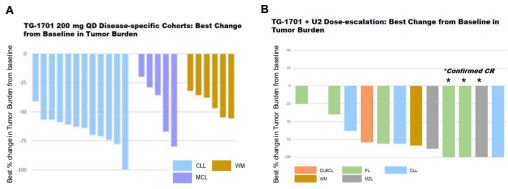


Fig. 5 (A) ADCC and ADCP *in vitro* results were confirmed *in vivo* in two MCL xenograft models, REC-1wt and UPN-1res. In both models, TG-1701 was administered either alone or together with a combination of ublituximab and umbralisib. In REC$^{GFP+Luc+}$ model, ublituximab and umbralisib exhibited similar effect (77% and 81% TGI, respectively) and no antagonistic effects were detected (76% TGI for the combination U2, data not shown). The triad combination of TG-1701 (54% TGI) and U2 (76% TGI) was more potent (88% TGI) than both treatments separately. The combination TG-1701 and umbralisib (87% TGI) showed similar tumor growth inhibition suggesting that blocking both BTK and PI3K slightly increase efficacy (87% TGI umbralisib alone, vs 87% TGI with TG-1701, data not shown). (B) In the UPN-1res model where TG-1701 had only a modest activity (17% TGI), the U2 arm showed a much stronger activity (55%) suggesting that resistances can be defeated using combination strategies. Here the triple combination, TG-1701+U2 showed a TGI of 57%. (C) Tumor samples from UPN^{Res} xenograft were studied to explore the interleukin signature and infiltration of NK cells as a mechanism of action. The addition of U2 to TG-1701 increased the pro-immune signature.

RESULTS #5: TG-1701, currently tested in CLL and NHL patients, show activity alone or in combination



Chan Y. Cheah et al, 25th Congress of the European Hematology Association (EHA), 12 June 2020. Abstract Code: EP705.

Fig. 6 (A) In the monotherapy dose expansion cohort in which TG-1701 was administered at 200mg, 25 patients were evaluable for efficacy with a 92% overall response rate (ORR) observed in CLL patients (n=12), a 33% ORR in MCL patients (n=6), and a 86% ORR in WM patients (n=7).

(B) The combination of TG-1701 plus U2 has demonstrated encouraging clinical activity with a 77% ORR across all disease types (n=13), including complete responses in three patients; dose escalation continues.

CONCLUSIONS:

- TG-1701 is a novel irreversible BTK inhibitor more selective and as active as ibrutinib in NHL models with BTK^{wt}
- When compared to ibrutinib, TG-1701 used at high doses retained notable antitumor activity in MCL cells with BTK^{C481S} mutation, while it did not show superior activity than the first-in-class BTKi in *in vitro* and *in vivo* models of ibrutinib-resistant MCL with constitutive activation of the non-canonical NFkB pathway.
- Combinations have been shown to overcome resistances in various diseases. Here, we explored the combination of TG-1701 with the novel, glycoengineered, CD20 antibody ublituximab and the PI3Kδ inhibitor umbralisib. We first showed that TG-1701, in contrast to ibrutinib, does not block neither ublituximab-driven ADCC nor ADCP in vitro. *In vivo* xenograft studies suggested that TG-1701 synergized with ublituximab and umbralisib. Part of the mechanism is related to the pro-immune interleukin signature and infiltration of NK cells in the tumor.
- TG-1701 is currently tested in clinical trial alone or in combination with umbralisib and ublituximab. Preliminary data showed a strong activity of the tri-therapy.
- The data presented here shed light on the scientific rationale of these early clinical data.

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