PF638

TG-1701 is a novel, orally available and covalently bound BTK inhibitor

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Background

- Background: Targeting Bruton's tyrosine kinase (BTK), an essential component of the BCR signaling pathway, has been demonstrated to be an effective treatment option for B-cell malignancies and autoimmune diseases. However, new BTK inhibitors are needed to allow for better safety and efficacy as a single agent and in combination with other agents.
- Aims: Herein we present TG-1701, a novel, orally available and covalently-bound BTK inhibitor that exhibits unique pharmacologic properties compared to prior BTK inhibitors.
- Methods: TG-1701 was evaluated and compared to ibrutinib and/or acalabrutinib in numerous enzyme based, cell-based, and animal models
- Results: TG-1701 and ibrutinib have comparable IC50s against BTK (3 nM and 1 nM respectively). TG-1701 exhibits superior selectivity to BTK compared to ibrutinib in an in vitro whole kinome screening (DiscoverX, San Diego, CA):

IC50 (nM)	BTK	HER2	ITK	HER4	CSK	EGFR
TG-1701	3	> 3000	> 3000	147	347	270
ibrutinib	1	36	62	4	57	2

- In addition, TG-1701 is 61-fold less active on EGFR compared to BTK with a Kd of 270 nM and 4.4 nM respectively. Ibrutinib, however, is only 6.7-fold less active on EGFR compared to BTK with a Kd of 2 nM and 0.3 nM respectively.
- TG-1701 inhibited the growth of the follicular lymphoma DOHH-2, mantle cell lymphoma Mino and DLBCL SU-DHL-6 cell lines with GI50% of 369, 449 and 313 nM respectively. TG-1701 inhibited IgM-activated BCR pathway in DOHH-2 cells, in particular the phosphorylation of BTK, PLCγ2 and ERK1/2. In a cell-based assay, TG-1701 blocked IgM-dependent CD69 expression, adhesion of JEKO cells to VCAM-1, and CXCL12-dependent migration.
- A fluorescent BTK-occupancy assay was developed and validated in vivo, in the spleen of mice, where BTK was found to be completely occupied after administration of a single dose of TG-1701 at 12.5 mg/kg. In vivo, the anti-tumor efficacy of TG-1701 was assessed in several lymphoma xenograft models, e.g. SU-DHL-6 (GCB-DLBCL), Mino (MCL), and OCI-Ly10 (ABC-DLBCL), where TG-1701 showed potent anti-tumor activity equivalent to or greater than ibrutinib and similar to the recently approved BTK inhibitor, acalabrutinib. In addition, the pharmacokinetic profile of TG-1701 allows for a once a day dosing.
- Summary/Conclusion: TG-1701 is a novel and highly-selective, irreversible BTK inhibitor with potent in vitro and in vivo activity. TG-1701 is currently being tested in a phase 1 dose escalation study.

In vitro selectivity

Kinase inhibition IC50 (nM)

	acalabrutinib	TG-1701	zanubrutinib	ibrutinib
BTK	5.1	3	0.22	1.5
TEC*	93	4	1.9	7
BMX#	46	~1000		0.8
TXK	368	136		2
ERBB2	1000	> 3000	661	36
EGFR	> 1000	270	660	2.0
ITK	> 1000	> 3000	30	62
JAK3	> 1000	> 3000	200	32
BLK#	> 1000	~1000		0.1

***TEC**: TEC knock-out study have shown that Tec-deficient mice developed normally and had no major phenotypic alterations of the immune system (Ellmeier et al, J. Exp. Med. 2000).

#BMX, BLK : TG-1701, 1uM, inhibited 54% of BLK and 72% of BMX activity

Acalabrutinib and ibrutinib data are from **Covey,** et al. *Cancer Res.* 2015; 259 and **Byrd JC**, Harrington B, O'Brien S, et al. *N Engl J Med* 2016;374:323-32.

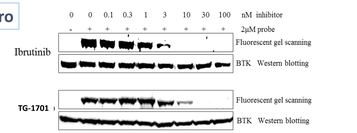
TG-1701 data are from EuroFins/Discoverex KinomeSCAN platform

Zanubrutinib (BGB31111) data are from presentation from Dr. Con Tam, 2017.

In vitro and *in vivo* Pharmacology

Occupancy assay

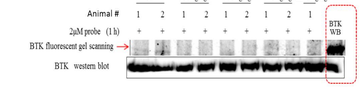
In vitro



Occupancy of BTK by TG-1701 was measured in DOHH-2 cells using a fluorescent analogue probe. Fluorescent signals of BTK-specific bands decreased in a dose-dependent manner after TG-1701 treatment. TG-1701 at 30 nM achieved complete occupancy on BTK.

DOHH-2 cells were treated with T-1701 or Ibrutinib for 1 hour and washed. The fluorescent probe (2 μM) was incubated for 1h and washed. Cells were harvested and lysed with 1 x SDS loading buffer and the cell lysate heated at 95 °C for 5 min. The resulting protein samples were resolved in an 8% SDS-PAGE. Fluorescent signals on the gel were detected using fluorescent gel imaging system and then proteins were transferred onto a PVDF membrane and total BTK proteins were detected by HRP-based western blotting using anti-BTK antibody.

In vivo

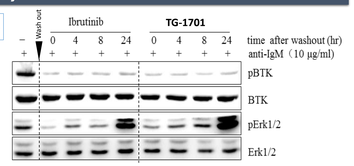


Binding of BTK with the fluorescent probe in splenocytes of Balb/c mice dosed with TG-1701 (12.5, 25, 50 mg/kg) for 2 hours was significantly inhibited. TG-1701 occupied 100% of the BTK protein in the splenocytes at the lowest tested dose (12.5 mg/kg). Ibrutinib (50 mg/kg) also showed complete occupancy on BTK protein after singe oral gavage.

Balb/c mice (n=2) were treated with increasing doses of TG-1701 by oral gavage. After 2h, mice were sacrificed and splenocyte isolated. Cells were harvested, lysed and lysates processed as described above.

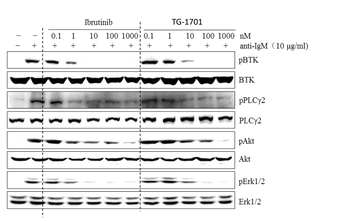
Pathway inhibition

In vitro



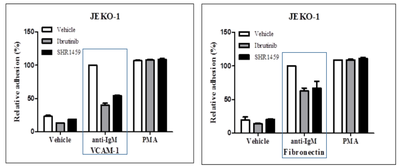
TG-1701 (100 nM) significantly suppressed phosphorylation of BTK and the downstream kinase Erk1/2 in DOHH-2 cells. After washout, TG-1701 retained its inhibitory effect on BTK signaling for up to 24 hours. The results demonstrated that TG-1701 was an irreversible inhibitor of BTK with a long-lasting inhibitory effect.

In vitro



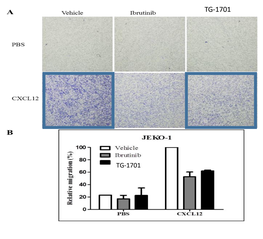
The inhibitory effects of TG-1701 on the BTK signaling pathway was determined on DOHH-2 cells incubated with increasing concentrations of TG-1701 for one hour. TG-1701 effectively blocked the activation of BTK signaling pathway stimulated by anti-IgM antibody, as shown by the dose-dependent inhibition on phosphorylation of BTK and the downstream enzymes (PLCγ2, Akt and Erk1/2). The inhibitory activity of TG-1701 on BTK signaling pathway was equivalent to that of Ibrutinib.

In vitro pharmacodynamic activity of TG-1701



Inhibition of VCAM- and fibronectin-dependent adhesion induced by BCR signaling and migration towards CXCL12 were examined in JEKO-1 cells treated with TG-1701 for one hour.

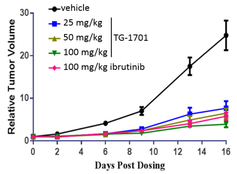
100 nM TG-1701 inhibited adhesion to VCAM-1 (50%) and Fibronectin (30%) in JEKO-1 cells stimulated with anti-IgM (BCR). The inhibitory effects were comparable to Ibrutinib. Neither of the drugs had effects on adhesion induced by PMA. This is possibly due the fact that PMA stimulates adhesion through PKC downstream of BTK, indicating that TG-1701 and Ibrutinib selectively inhibited cell adhesion triggered by BCR signaling.

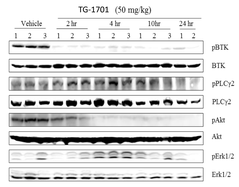


- CXCL12 guides lymphocyte migration through the PI3K-BTK-PKC or PI3K-Rac-JNK signal transduction pathways after binding to its receptor.
- CXCL12 promotes migration of JEKO-1 (A, lower left micrograph) and 100 nM TG-1701 inhibited migration of JEKO-1 cells towards CXCL12 (~40%, lower right micrograph), indicating that TG-1701, like Ibrutinib, partially inhibited cell migration mediated by CXCL12.
- These results demonstrated that TG-1701 selectively inhibited cell adhesion stimulated by BCR signaling and migration mediated by CXCL12.

In vivo anti-tumor and anti-inflammatory activity

In vivo xenograft DOHH-2 model : Efficacy and PD study

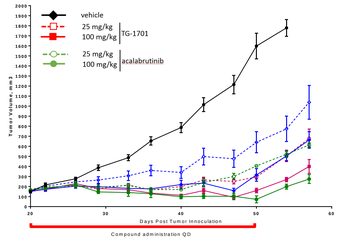




A TG-1701 dosed orally once a day at 25, 50 and 100 mg/kg inhibited tumor growth in DOHH2 tumor-bearing mice. TG-1701 and ibrutinib, in this model, demonstrated comparable anti-tumor activities, and all treatments were well tolerated with no body weight loss (data not shown).

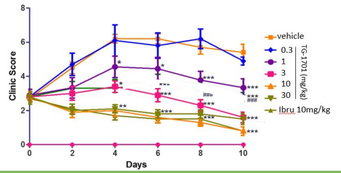
B Following single oral gavage of TG-1701 (50 mg/kg) phosphorylation of BTK and Akt in DOHH-2 tumor tissue was significantly inhibited from 2 to 24h post dose. However, phosphorylation level of PLCγ2 and Erk1/2 increased first, with peak at 4 hours, before decreasing 10 hours post-dose. The temporary increase in phosphorylation levels of PLCγ2 and Erk1/2 proteins might be due to the negative feedback of BTK inhibition. The inhibitory pattern of TG-1701 (50 mg/kg) on BTK, PLCγ2, AKT and Erk1/2 phosphorylation was like that of Ibrutinib (not shown).

In vivo xenograft OCI-LY-10 model : comparison with acalabrutinib



- OCI-LY-10 is an ABC-DLBCL cell line, L265P Myd88 mutant, sensitive to Ibrutinib.
- Both acalabrutinib and TG-1701 were dose by oral gavage, once day for 28d.
- The activity observed for acalabrutinib with a Day 43 median ΔT/ΔC of 15% and a TGD of 22.7 days, was similar to that observed with TG-1701
- Treatments against established OCI-Ly-10 xenograft model were well tolerated producing no treatment related mortality or body weight loss.

In the CIA mouse model, TG-1701 reduced arthritis clinical score



In Collagen-induced arthritis mice, TG-1701:

- reduced clinical score of arthritis at 1, 3, 10 or 30 mg/kg;
- ameliorated plantar swelling at 3, 10 or 30 mg/kg;
- reduce serum IL- 1β, IL-6 and TNFα levels at 0.3, 1, 3, 10 or 30 mg/kg in a dose-dependent manner;
- significantly reduce pathological score of ankle joint at 10 mg/kg.
- At the same dose of 10 mg/kg, efficacy of TG-1701 was comparable to Ibrutinib.

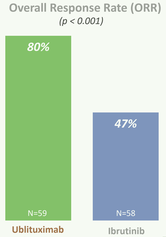
Conclusions

- TG-1701 is a novel, specific and covalent BTK inhibitor, more selective than ibrutinib toward a set of kinases including EGFR
- Occupancy assays in vitro and in vivo suggest that 100% occupancy can be reached using low dose in human dose escalation clinical trial.
- TG-1701 inhibited the phosphorylation of BTK and other kinases downstream the BCR pathway, demonstrates a strong growth inhibitory activity against a set of lymphoma cell lines (data not shown) and inhibits CXCL12-dependent cellular adhesion.
- TG-1701 demonstrated similar antitumor efficacy to ibrutinib and acalabrutinib.
- PK profile allows for a once-a-day dosing, TG-1701 is not a CYP inhibitor, and possess a favorable profile for combinations (data not shown)
- In clinic, a phase 1 dose escalation has started in China
- TG-1701 will be tested in combination with several TG assets including ublituximab and umbralisib.

Clinical perspective

Potential for TG-1701 Use in Combination

Ublituximab



- Randomized patients with high-risk relapsed or refractory CLL: centrally confirmed presence of del17p, del11q, and/or TP53 mut
- Well tolerated with safety profile consistent with recently published Phase 2 data
- Primary endpoint met with all responses assessed by independent central review per iwCLL 2008 guidelines
- Median follow-up was ~12 months

GENUINE TG THERAPEUTICS GLYCOPROTEIN ANTI-CD20 • UBLITUXIMAB PHASE 3 TRIAL IN HIGH-RISK CLL

Sharman et al., ASCO 2017

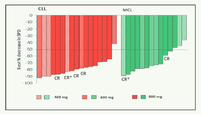
Umbralisib

DANA-FARBER CANCER INSTITUTE | BRIGHAM AND WOMEN'S HOSPITAL

Updated Results of a Multicenter Phase I/IB Study of Umbralisib (TGR-1202) in Combination with Ibrutinib in Patients with Relapsed or Refractory MCL or CLL

Results

Updated Efficacy Analysis (n=31)

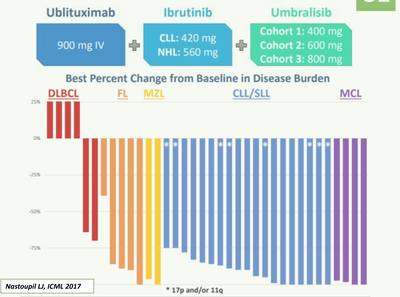


CLL (n=17)	MCL (n=14)
• ORR: 16/17 (94%) -PR or PR-L: 15/17 (88%) -CR: 1/17 (6%), 3 other pts with radiographic CR • All 3 pts with prior PI3Ki and 1 of the 2 pts with prior ibrutinib responded	• ORR: 11/14 (79%) • PR: 10/11 (71%) • CR: 1/11 (9%), 1 other pt with radiographic CR • Marked clinical benefit observed in 2 additional pts

*meets formal disease–specific criteria for CR

Davids M, ICML 2017

U2



Nastoupil LJ, ICML 2017

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