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TG-1601 is a novel BET inhibitor with strong binding affinity and long-lasting effect in preclinical models

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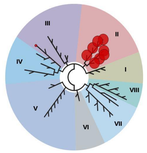
Background

- BET (bromodomain and extra-terminal) proteins bind to acetylated lysine residues on chromatin and participate in the regulation of gene transcription. Inhibition of BET protein binding to chromatin with small molecules selectively suppresses the transcription of a set of oncogenes, including MYC and BCL-2.
- TG-1601 (aka CK-103) is a novel, selective and potent small molecule inhibitor of BET bromodomains. TG-1601 binds to the first and second bromodomains (BD1, BD2) of the BET protein family, BRD2, BRD3, BRD4, and BRDT, with Kd values ranging from 0.5 nM to 9.1 nM. MYC protein expression is strongly inhibited in the MV4-11 cancer cell line with an EC50 of 5 nM, with GI50 comprised between 15 nM and 85 nM in a variety of leukemia and myeloma cancer cell lines, indicating potent inhibition of cell proliferation.
- Time course and dose-response studies conducted in mice bearing subcutaneous MV4-11 xenografts showed that MYC protein was undetectable 3 hours following a single 25 mg/kg oral dose, with a TG-1601 tumor concentration of 5 µM achieved. Interestingly, at 24h post-dose, while TG-1601 is cleared from the tumor, MYC protein level remains below 40% of its initial level, indicating a long-lasting effect pharmacodynamic of TG-1601, potentially attributable to enhanced binding affinity compared to earlier generation molecules.
- In agreement with this long-lasting effect, efficacy studies in MV4-11 tumor-bearing mice, dosed with a 20 mg/kg/day PO regimen interrupted by increasing drug holiday periods, showed that drug holidays of 2, 3 and 4 days per week only modestly affected efficacy (3%, 15% and 12% TGI respectively), suggesting discontinuous dosing of TG-1601 in clinic may not significantly impact efficacy.

Kd (nM)
TG-1601 and two related BET inhibitors

bromodomain	TG-1601	JQ1	OTX-015
BRD2(BD1)	8.2	57	20
BRD2(BD2)	0.65	35	3
BRD3(BD1)	4	32	12
BRD3(BD2)	0.46	38	2
BRD4(BD1)	11	31	13
BRD4(BD2)	0.81	29	4.9
BRDT(BD1)	9.1	120	28
BRDT(BD2)	2.2	51	10
CREEBBP	640	> 3000	> 3000
EP300	660	> 3000	> 3000

Binding constants were assessed using the BROMOscan platform from Discoverx. The assay includes trace bromodomain concentrations (<0.1 nM) and thereby report true thermodynamic inhibitor Kd values.



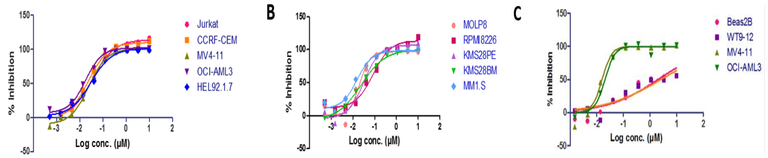
Selectivity tree of TG-1601 (1 µM) on 32 bromodomain-containing family members

Using the assay presented above, the displacement of the bromo-probe by 1uM TG-1601 was assessed on 32 bromodomain containing proteins.

On the phylogenic tree (TreeSpot) presented here, TG-1601 only binds the members of the BET family.

The diameter of each red sphere is inversely proportional to The binding of the bromo-probe (that has not been displaced by TG-1601)

In vitro cytotoxic activity



Objective: The objective of this study was to characterize, *in vitro*, the 72 hrs cytotoxic activity of TG-1601 against a panel of leukemia, multiple myeloma and normal cell lines.

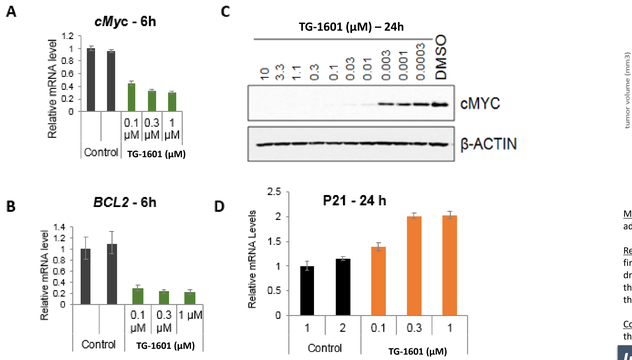
Methods: 5 leukemia, 5 multiple myeloma and 2 normal (transformed) cell lines were seeded in 96 wells at different densities, and incubated with increasing concentrations of TG-1601 for 72 hrs.

Results: **A -** The growth of 5 leukemia cell lines, Jurkat, CCRF-CEM, MV4-11, OCI-AML3 and HEL92.1.7 were inhibited with EC_{50} values of 35 nM, 24 nM, 24 nM, 18 nM and 31 nM respectively.
B -The growth of 5 multiple myeloma cell lines, MOLP8, RPMI8226, KMS28PE, KMS28BM and MM1s was inhibited with EC_{50} of 21 nM, 85 nM, 32 nM, 24 nM, and 15 nM respectively.
C - The growth of two normal (transformed) cell lines (Beas2B and WT9-12) was not inhibited more than 50% at 10 µM TG-1601.

Conclusion: TG-1601 is a potent inhibitor of hematologic tumor cell growth, with all EC_{50} below 100 nM, but was not potent against normal (transformed) cell lines, suggesting, *in vitro*, a potential therapeutic window.

In vitro and *in vivo* Pharmacodynamic activity

In vitro pharmacodynamic activity of TG-1601

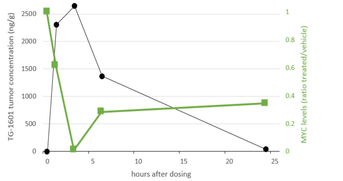


Methods: MV4-11 cells were seeded in 96 wells and incubated with increasing concentrations of TG-1601 for 6 or 24 hrs. mRNA or protein were extracted from cell lysate, and qPCR or Western blot were run. GADPH was used as a loading control.

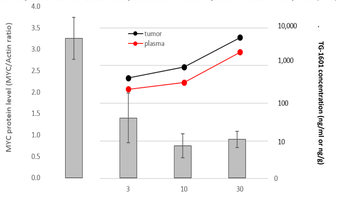
Results: Consistent with published data, the BET inhibitor TG-1601 induced the rapid down-regulation of MYC and BCL2 mRNA in the MV4-11 AML cell line (more than 60% inhibition compare to control, (**Figure A and B**) at doses that induced cell growth inhibition. At 24 hrs, MYC protein expression was strongly inhibited (EC_{50} = 5 nM, **Figure C**). In addition, the tumor suppressor gene p21, a well-known MYC target, was upregulated upon MYC suppression by TG-1601 (**Figure D**) by 1.3, 2.0 and 2.0-fold when cells were treated with TG-1601 at 0.1, 0.3 and 1 µM, respectively.

Conclusions: Consistent with published data, the BET inhibitor TG-1601 induced in cells the rapid down-regulation of MYC and BCL2 and an increase of p21 mRNA.

In vivo pharmacodynamic activity of TG-1601



MV4-11 tumor-bearing mice were dosed with 25 mg/kg TG-1601 once and tumor removed at 1 h, 3 hrs, 6 hrs and 24 hrs post-dose. Western blots run on tumor lysates, and immunoblot scanned and quantified. TG-1601 was quantified in tumor lysate for each time point (n=3).



MV4-11 tumor-bearing mice were dosed with 3, 10 or 30 mg/kg TG-1601 once and tumor were removed at 3 hrs post-dose. Western blots run on tumor lysates, and immunoblot scanned and quantified. TG-1601 was quantified in tumor lysate and plasma samples for each time point (n=3).

Methods: In both studies, athymic nude mice were inoculated subcutaneously with MV4-11 cancer cells to establish the tumor model. The doses administered were 3, 10 and 30 mg/kg once (lower panel) or 25 mg/kg, once (upper panel). In each study, TG-1601 doses were administered by oral gavage. C-Myc protein expression was monitored using Western blotting and GADPH was used as a loading control. TG-1601 levels were assessed in plasma and tumor (lower panel) or tumors (upper panel).

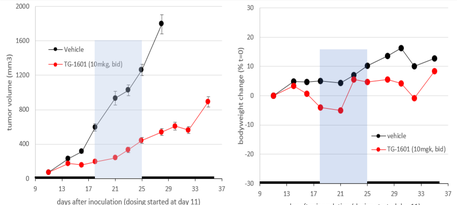
Results: In the first experiment (lower panel), c-Myc protein levels were decreased by 57%, 77% and 72% 3 hrs after 3, 10 and 30 mg/kg TG-1601 oral gavage, respectively. At these doses TG-1601 tumor concentration was 465, 915 and 5502 ng/mL respectively. At these doses, CCR2 mRNA levels were decreased by 52.9%, 50.3% and 68.3% respectively (data not shown).

In a second experiment (upper panel), c-Myc protein levels were decreased by 38%, 99%, 72% and 65% 1 h, 3 hrs, 6 hrs and 24 hrs after a single 25 mg/kg TG-1601 dose, respectively. At these times TG-1601 tumor concentrations were 2315, 2646, 1372 and 38 ng/mL respectively.

Conclusion: In good agreement with the proposed mechanism of action, c-Myc levels were rapidly decreased in the tumor (and was undetectable at 3 hrs) after a single dose of TG-1601 by oral gavage. Interestingly the level of c-Myc did not come back to its original levels at 24 hrs, even though TG-1601 was barely detectable (tumor concentration was 38 nM) in the tumor. This may suggest a long-lasting effect of the drug in this model.

In vivo anti-tumor activity

In vivo anti-tumor activity in MM1s multiple myeloma model



Left panel: Tumor volume; The black solid bar on the X axis indicated the period of treatment. Right panel: Body weight changes compare to pre-dose weight.

The blue rectangles represent the interruption of dosing from Day 18 to Day 25 to allow body weight to recover.

Methods: SCID/Beige mice were inoculated subcutaneously with MM1s cancer cells to establish the tumor model. The TG-1601 dose administered was 10 mg/kg, bid.

Results: TG-1601 (10 mg/kg, bid) reduced tumor volume by 70% at day 28, 17 days after the start of treatment (**left panel**). After the first week of treatment, average body weight was reduced by 5%. Treatment was interrupted for 1 week and then resumed. During this drug holiday period (blue rectangles), tumors in the treated arm did not grow back as fast as in the vehicle arm, suggesting that this therapeutic window did not impact efficacy. During drug holiday, mice recovered and body weight of the treated group was similar to the vehicle treated group.

Conclusion: TG-1601 demonstrate activity in a multiple myeloma xenograft model. A therapeutic window of one week did not impact the efficacy of the treatment.

In vivo anti-tumor activity in MV4-11 AML model

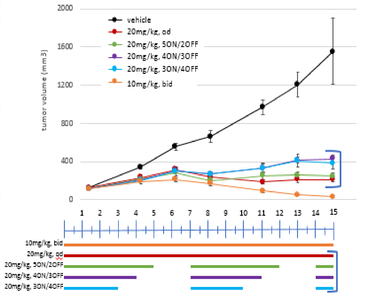
Methods: TG-1601 was dosed 20 mg/kg/day, either continuously or using a schedule that included 2, 3 and 4 days off per week. TG-1601 was also dosed 20 mg per day, using a 10 mg/kg bid schedule.

Results: TGI% were 94%, 91%, 78% and 82% in groups treated with 20 mg/kg continuously with no drug holiday, or with 2, 3 and 4 days off per week, respectively.

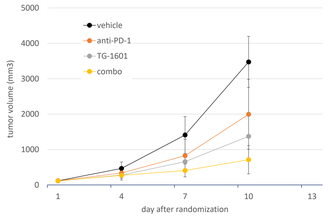
Results suggest that drug holidays can be applied without losing efficacy. In addition, the 10 mg/kg twice a day schedule was more efficient than the 20 mg/kg once a day, as 6/6 mice were tumor free at day 15 in the 10 mg/kg arm, whereas no mice were tumor free in the 20 mg/kg dosing.

Efficacy (TGI%) were correlated in all arms with a decrease of MYC mRNA level of 60% or more (data not shown).

Conclusion: Orally administered TG-1601 demonstrated significant anti-tumor activity at 20 mg/kg/day. Insertion of drug holidays did not significantly affected the efficacy of TG-1601 suggesting that treatment interruptions during clinical trial could be beneficiary by increasing the therapeutic window.



In vivo anti-tumor activity in combination with anti-PD-1 antibody (B16 syngeneic model)



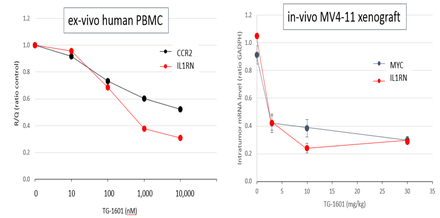
Methods: BALB/c immunocompetent mice were inoculated subcutaneously with the mouse B16F10 melanoma cell line in a syngeneic mouse model.

Results: the combination of TG-1601 with an anti-PD-1 antibody was tested in the B16F10 melanoma syngeneic model. TGI was 42%, 50%, and 54% for the anti-PD-1 antibody, TG-1601 or the combination, respectively.

Conclusion: The combination of an anti-PD-1 antibody with TG-1601 demonstrate a synergistic antitumor activity.

Pharmacodynamic markers

In vivo and *ex-vivo* validation of CCR2 and IL1RN



Left panel: TG-1601 was spiked in whole blood at increasing concentrations (n=4). The control samples were spiked with 0.5% DMSO, final. RQ: ratio of target gene / GADPH.
Right panel: In an *in vivo* experiment, TG-1601 3, 10, 30 mg/kg was orally administered to MV4-11 tumor-bearing mice, and intra-tumoral MYC and IL1RN mRNA quantified by qPCR (n=3).

Methods: TG-1601 (0.01, 0.1, 1 and 10 µM) was spiked in whole blood of 4 healthy volunteers and the RNA Later solution was added 3 hrs after dosing to stabilize mRNA. CCR2 and IL1RN mRNA were quantified using qPCR.

Results: IL1RN and CCR2 mRNA expressions are both under BET control, and have been shown to be decreased under BET inhibitor treatment. CCR2 was decreased by 50%, 40% and 30% and IL1RN by 72%, 63%, and 35% when TG-1601 was spiked at 10 nM, 100 nM, 1 µM and 10 µM respectively (left panel). In another experiment, in the MV4-11 xenograft model, TG-1601-induced inhibition of the intratumor level of IL1RN and MYC were very comparable (**Figure 4**, right panel).

Conclusions: IL1RN and CCR2 mRNA detection directly in the blood of patients treated with TG-1601 can serve as a surrogate pharmacodynamic marker during the dose-escalation Phase 1 clinical trial. These two genes have been used in clinic to assess BET inhibitor target engagement. Here we show that TG-1601 blocked both mRNA expression in a dose-dependent manner, and MYC and IL1RN imRNA expression are similarly inhibited by TG-1601 *in vivo*.

Conclusions

- TG-1601 is a novel and potent BET inhibitor that specifically inhibits the binding of the BET sub-family of bromodomain-containing protein family
- TG-1601 potently inhibits cell growth of various multiple myeloma and lymphoma cell lines in vitro, but does not affect the growth of normal (transformed) cell lines.
- TG-1601 inhibits MYC expression:
  - In vitro, TG-1601 potently inhibit Myc expression at the RNA and protein levels
  - In vivo, TG-1601 totally inhibits Myc protein expression at 3h post dose. Interestingly the level of c-Myc did not come back to its original levels at 24 hrs, even though TG-1601 was barely detectable in the tumor. This may suggest a long-lasting effect of the drug in this model.
- In different in vivo xenograft models, TG-1601 potently inhibits tumor growth and drug holidays do not alter its anti-tumor activity that treatment interruptions during clinical trials could be beneficiary by increasing the therapeutic window.
- TG-1601 showed combinatorial effects in an in vivo model with anti-PD-1 antibodies. Clinical trials will be focused on a potential synergism between TG-1601 and other drugs in the TG pipeline (e.g. anti-PDL-1, BTK inhibitor, anti-CD20 antibody (ublituximab) or PI3K inhibitor (umbralisib).
- As an important part of the phase 1 dose-escalation, surrogate markers (e.g. CCR2 and IL1RN mRNA levels) will be tested to define the Pharmacologically Active Dose.

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