Preclinical Characterization of a Novel Non-Cyclic Dinucleotide Small Molecule STING Agonist with Potent Anti-Tumor Activity in Mice

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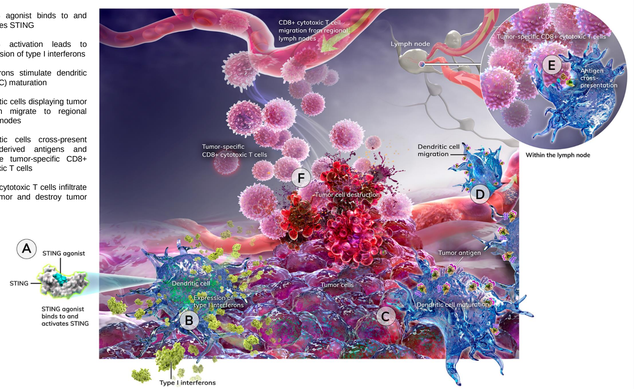
STING Bridges Innate and Adaptive Anti-Tumor Immunity

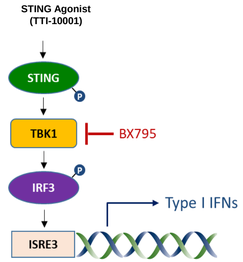
A. STING agonist binds to and activates STING
B. STING activation leads to expression of type I interferons
C. Interferons stimulate dendritic cell (DC) maturation
D. Dendritic cells displaying tumor antigen migrate to regional lymph nodes
E. Dendritic cells cross-present tumor-derived antigens and activate tumor-specific CD8+ cytotoxic T cells
F. CD8+ cytotoxic T cells infiltrate the tumor and destroy tumor cells



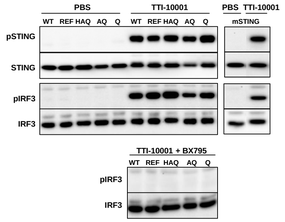
- STING (stimulator of interferon genes) has emerged as an attractive cancer immunotherapy target due to its key role in induction of type I interferons (IFNs) and other pro-inflammatory cytokines that promote tumor-specific antigen cross-presentation and effective T cell priming
- Currently, all known STING agonists in clinical trials are based on cyclic dinucleotide (CDN) scaffolds, however, these high molecular weight synthetic CDNs often display limited potency, cellular permeability, stability, and a short tumor retention time
- In this study we present TTI-10001, a novel non-CDN small molecule STING agonist with favorable drug properties and potent anti-tumor activity in mice

TTI-10001 Activates the STING Signaling Pathway

A) STING signaling pathway

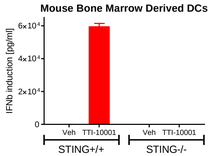


B) TTI-10001 dependent phosphorylation of STING/IRF3



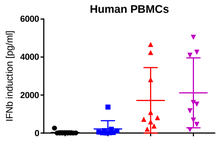
Total or phosphorylated STING (pYS366) and IRF3 (pYS396) were detected by western blot of HEK-293T cells stably transfected with individual STING alleles, after 4 hours incubation with 10 μM TTI-10001 (+/- pretreatment with TBK1 inhibitor BX795, 10 μM)

C) Mouse IFNβ induction by TTI-10001



IFNβ was measured after 4 hours incubation with 10 μM TTI-10001. STING-/- DCs are from Goldenticket mice lacking functional STING

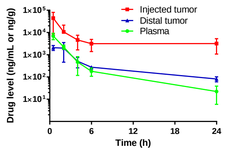
D) Human IFNβ induction by TTI-10001



IFNβ was measured after 4 hours incubation with the indicated concentrations of TTI-10001

TTI-10001 Achieves High *In Vivo* Exposure and Durable Tumor Retention

A) TTI-10001 *in vivo* drug levels



B) PK parameters of TTI-10001

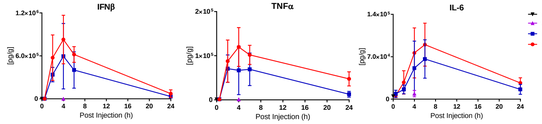
Tissue	Apparent $t_{1/2}$ (h)	C_{max} (ng/ml)	$AUC_{0\text{-}tlast}$ (h*ng/ml)
Injected Tumor	NC*	44,647	143,225
Distal Tumor	8.4	2,077	9,374
Plasma	5.02	7,007	12,240

*not calculable, projected $t_{1/2}$ >8

A) and B) C57BL/6 mice bearing dual flank MC38 tumors were injected with 100 μg TTI-10001 into right flank tumors only

TTI-10001 Induces Pro-inflammatory Cytokine Expression and Promotes T Cell Activation *In Vivo*

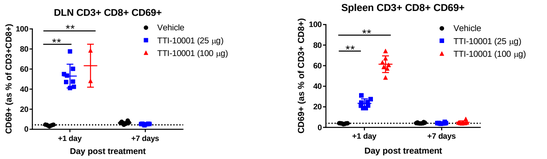
A) Cytokine induction by TTI-10001 in MC38 injected tumors



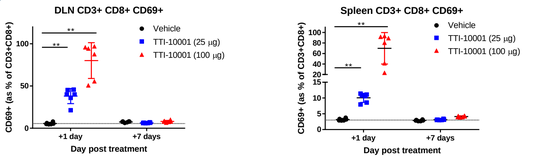
MC38 tumors were injected with TTI-10001 (25 or 100 μg) or vehicle control. Tumors were harvested at several time points post injection and cytokine levels were measured by cytometric bead array

B) Activation of CD8+ T cells in tumor draining lymph node (DLN) or spleen

MC38

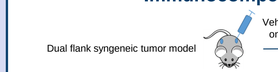


A20

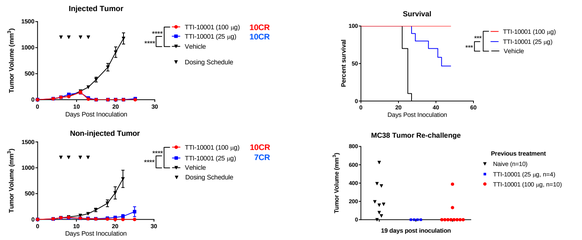


*Tumor nearby DLNs or spleens were collected 1 or 7 days after the third IT injection. CD3+ CD8+ cells were analyzed for expression of the T cell activation marker CD69. Statistical significance: T-test, ** p<0.01*

TTI-10001 Induces Tumor Regression in Two Immunocompetent Mouse Models

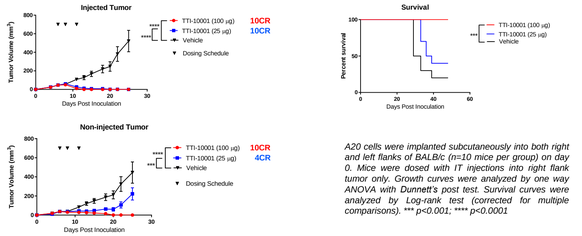


A) MC38 (colon adenocarcinoma) model



*MC38 cells were implanted subcutaneously into both right and left flanks of C57BL/6 (n=10 mice per group) on day 0. Mice were dosed IT into right flank tumor only. TTI-10001-treated mice free from tumors for 30 days were re-challenged with MC38 cells in the right flank. Naive C57BL/6 mice were inoculated with MC38 cells as control. Tumor re-growth was monitored for 19 days. Growth curves were analyzed by one way ANOVA with Dunnett's post test. Survival curves were analyzed by Log-rank test (corrected for multiple comparisons). CR: complete tumor regression; *** p<0.001; **** p<0.0001*

B) A20 (B-cell lymphoma) model



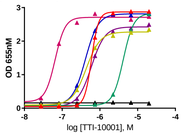
*A20 cells were implanted subcutaneously into both right and left flanks of BALB/c (n=10 mice per group) on day 0. Mice were dosed with IT injections into right flank tumor only. Growth curves were analyzed by one way ANOVA with Dunnett's post test. Survival curves were analyzed by Log-rank test (corrected for multiple comparisons). *** p<0.001; **** p<0.0001*

TTI-10001 is a Potent, Cell Permeable, Small Molecule Pan-STING Agonist

A) Key drug properties of TTI-10001

- Non-CDN based
- MW <500 Daltons
- tPSA: 85
- LogD: ~1
- Solubility: > 4mg/ml in water
- Permeability (Caco2):1.1x10^{-6}cm/s

B) Dose-dependent reporter activation by TTI-10001



C) TTI-10001 cellular potency summary

STING Allele	Allele Frequency (Humans)*	EC_{50} (μM)
Human STING-WT	57.9%	0.51 ± 0.08
Human STING-HAQ	20.4%	0.57 ± 0.10
Human STING-REF	13.7%	3.96 ± 0.66
Human STING-AQ	5.2%	0.49 ± 0.09
Human STING-Q	1.5%	0.64 ± 0.06
Mouse STING	-	0.12 ± 0.03

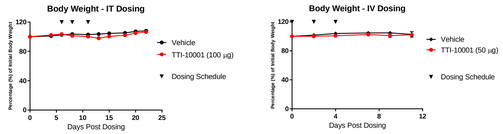
*B) and C) EC50s are based on reporter activation in HEK-293T stable cell lines transfected with individual human or mouse STING alleles. mSTING: mouse STING; * Yi, et al PloS One (2013)*

TTI-10001 Exhibits a Favorable Safety Profile and is Well Tolerated in Mice

A) *In vitro* ADME

Assay	CYP3A	CYP2D6	hERG
TTI-10001 IC_{50}	>30 μM	>30 μM	>100 μM

B) Mouse body weight after intratumoral (IT) or intravenous (IV) dosing



No deaths, body weight loss, or overt morbidity observed at the indicated doses

Conclusions

- **TTI-10001 is a novel, non-CDN, potent, small molecule pan-STING agonist**
- **TTI-10001 exhibits favorable potency, cell permeability, and tumor retention properties that could potentially overcome the common limitations of current CDN-derived STING agonists**
- **TTI-10001 is well tolerated in mice at relevant doses after IT or IV administration**
- **TTI-10001 induced durable complete regressions in both injected and distal tumors**
- **These data support further evaluation of TTI-10001 as a potential first-in-class small molecule STING agonist for cancer immunotherapy**