83. A novel small molecule agonist of STING, M04

(Oregon Health and Science University)

Asset Overview

Product Type	Small Molecule
Disease Area	Oncology
Indication	Ovarian cancer
Current Stage	Lead Optimization
Target	The adaptor protein Stimulator of Interferon Genes (STING)
MoA	A small molecule termed M04 that behaves as a novel agonist of human STING. Also, the molecule exhibits a differential ability to activate STING based on the allelic variant examined.
Brief Description	• STING functions to activate innate immune (especially type I interferon) responses, which can impair viral replication, and initiate adaptive immune processes including antibody and T cell-mediated activity against microbial pathogens and tumor cells. • Inventors have developed a novel small molecule agonist of STING, which may overcome some of the chemical liabilities of current STING inducers, as a potential anti-cancer therapy. • Testing in human peripheral blood mononuclear cells (PBMCs) taken from patients with pancreatic cancer found that treatment with the STING agonist induced several cytokines illustrative of the compound's ability to induce innate responses necessary for adaptive immunity. Testing in healthy human PBMCs also found the agonist was capable of facilitating maturation of antigen presenting cells (APC) and increased in the frequency of antigen-specific CD8+ T cells, indicating its adjuvant properties.
Intellectual Property	WO2021262878A1
Publication	Characterization of a Novel Compound That Stimulates STING-Mediated Innate Immune Activity in an Allele-Specific Manner. Front Immunol. (2020)
Inventors	Victor Defilippis, Jinu ABRAHAM, Sara BOTTO

Highlights

- M04 Is a Small Molecule That Activates Type I IFN Signaling in Human Cells
- M04-Mediated Innate Stimulation Requires Activation of TBK1 and IRF3
- M04 Does Not Stimulate Activation of Canonical NF-κB-Associated Transcription
- M04 Activates IRF3 and IFN-Terminal Signaling That Requires STING but Not MAVS, TRIF, or dsDNA PRRs
- M04 Induces Phosphorylation and ER-Golgi Trafficking of STING
- M04 Stimulatory Capacity Is Dependent on STING Polymorphic Variant
- M04 Triggers Expression of Human Dendritic Cell Maturation Markers
- M04 Enables T Cell Cross Priming
- The M04 Transcriptome More Closely Resembles That Induced by cGAMP Than by LPS

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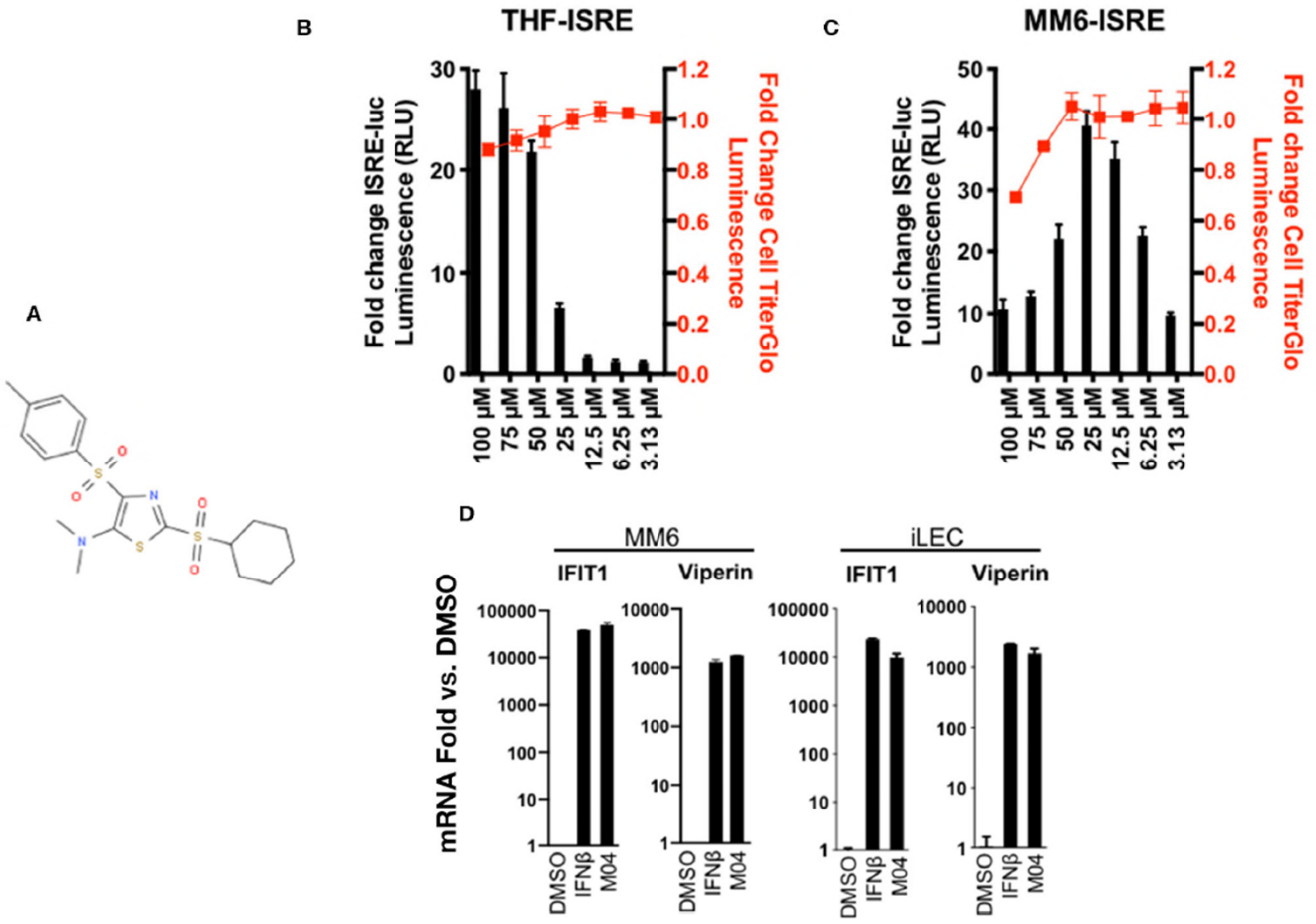
Key Data

A small molecule M04 that behave as an agonist of human STING



Dose-Dependent Activation of Type I Interferon-Mediated Signaling and Cytotoxicity of M04 in Human Cells. (A) Chemical structure of 2-(cyclohexylsulfonyi)-N,N-dimethyl-4-tosylthiazol-5-amine ("M04"); (B) ISRE-dependent expression of Luciferase (LUC) as well as relative cellular viability as determined by Cell Titer Glo in THF (B) and MM6 (C) cells exposed to M04 at indicated concentrations (µM) for 8 h (RLU) or 24 h (Cell Titer Glow). Values presented are mean fold change ± SD relative to cells treated with 1% DMSO (RLU; black bars; left y-axis). Cell viability data are expressed as relative signal detected in DMSO-treated cells (red squares; right axis). Values displayed are based on four replicates; (D) Fold changes of IFIT1 or Viperin mRNA relative to 1% DMSO treatment in immortalized lymphatic endothelial cells (iLEC) or MM6 following 8 h exposure to 1000U/mL IFNβ or 50 µM M04 as indicated. Presented values represent average ± SD mRNA fold changes relative to cells exposed to untreated cells from duplicate experiments.

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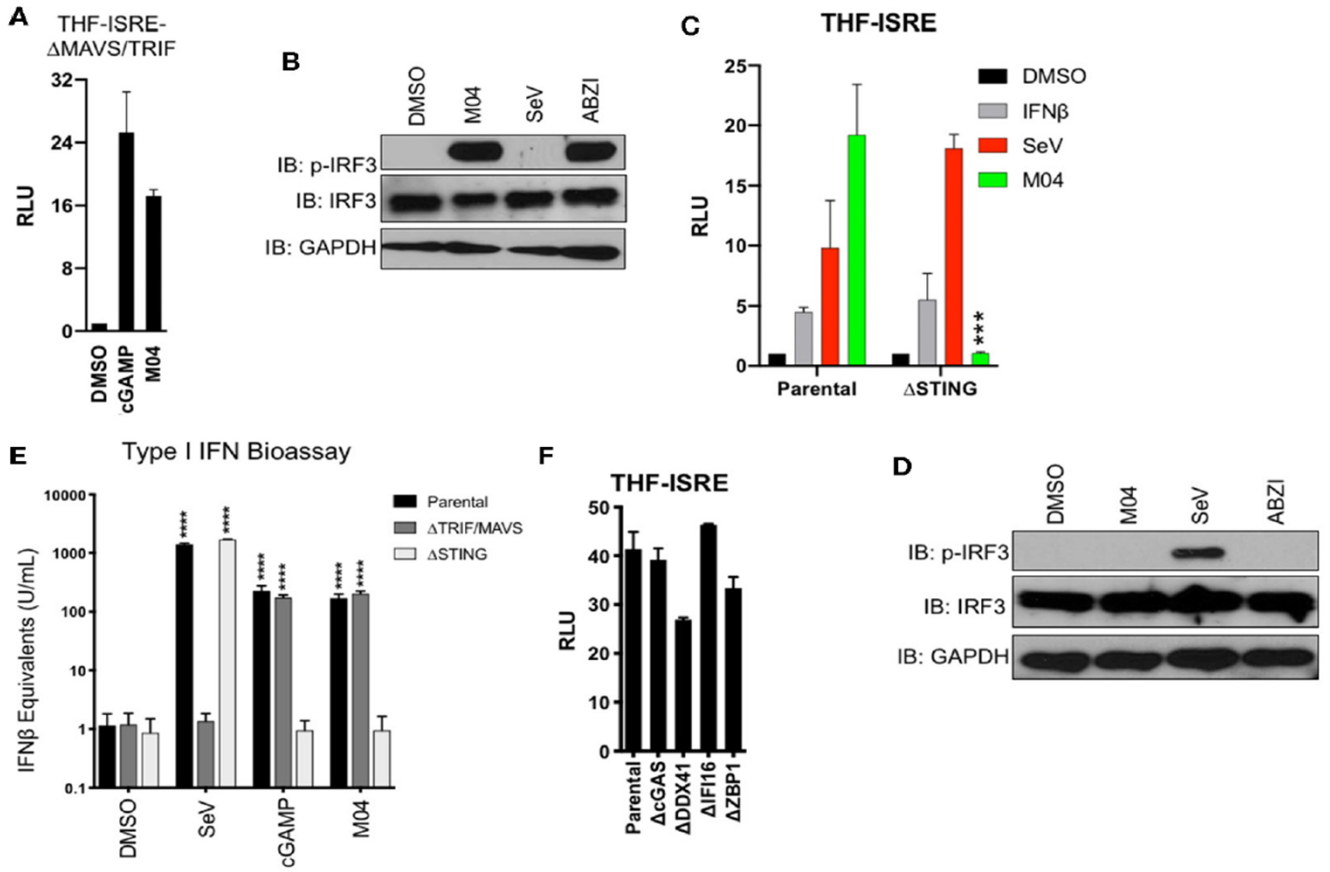

Innate Activation by M04 requires STING but not MAVS, TRIF, or cytosolic DNA PRRs. (A) Reporter assay illustrating IFN-dependent LUC induction in THF-ISRE-1MAVS/TRIF following overnight treatment with 1% DMSO, transfected cGAMP (10 µg/mL), or 75 µM M04. Data presented are mean ± SD relative luminescence units (RLU) using signal from DMSO-treated cells as the basis (n = 4 treatments); (B) Immunoblot showing phosphorylation status of IRF3 Ser386, total IRF3, and GAPDH in THF-ISRE-1MAVS/TRIF following 8 h treatment with 1% DMSO, 75 µM M04, 1,000 HAU/mL SeV or 25 µM ABZI as indicated; (C) Reporter assay illustrating IFN-dependent LUC induction in THF-ISRE-1STING following overnight treatment with 1% DMSO, 1,000 U/mL IFNβ, 1,000 HAU/mL SeV, or 75 µM M04. Data presented are mean RLU ± SD as described above; Student's T-test was used to compare RLU ***p < 0.001; (D) Immunoblot showing phosphorylation status of IRF3 Ser386, total IRF3 in THF-ISRE-1STING following 4 h treatment with 1% DMSO, 50 µM M04, 1,000 HAU/mL SeV or 25 µM ABZI as indicated; (E) Secretion of bioactive type I IFN from parental THF as well as THF-ISRE-1MAVS/TRIF and THF-ISRE-1STING treated in triplicate overnight with 1% DMSO, 1,000 HAU/mL SeV, transfected cGAMP (10 µg/mL), or 75 µM M04. Data are expressed as mean concentrations ± SD for IFNβ equivalent units. Statistical significance between treated and untreated cells of similar genetic background was calculated using Student's T-test. ****p < 0.0001; (F) Reporter assay from WT parental THF-ISRE cells as well as from cells from which indicated dsDNA-specific PRRs were deleted. Values presented are mean fold changes ± SD for duplicates relative to the value for DMSO-treated cells