**TITLE:** TAK-676: A Novel Stimulator of Interferon Genes (STING) Agonist Promoting Durable Interferon-Dependent Anti-Tumor Immunity in Preclinical Studies

**Authors:**

Elizabeth Carideo Cunniff1\*, Yosuke Sato1\*, Doanh Mai1\*, Vicky A. Appleman1, Shinji Iwasaki2, Vihren Kolev1, Atsushi Matsuda2, Judy Shi1, Michiyo Mochizuki2, Masato Yoshikawa2, Jian Huang1, Luhua Shen1, Satyajeet Haridas1, Vaishali Shinde1, Chris Gemski1, Emily R. Roberts1, Omid Ghasemi1†, Hojjat Bazzazi1‡, Saurabh Menon1, Tary Traore1§, Pu Shi1¶, Tennille D. Thelen1\*\*, Joseph Conlon1††, Adnan O. Abu-Yousif1, Christopher Arendt1, Michael H. Shaw1, and Masanori Okaniwa1

\*Authors contributed equally to this manuscript

**Affiliations:**

1Takeda Development Center Americas, Inc. (TDCA), Lexington, MA, USA

2Takeda Pharmaceutical Company, Ltd., Fujisawa, Kanagawa, Japan

†Current affiliation: Invicro, LLC, Needham, MA, USA

‡Current affiliation: Cytomx Therapeutics, South San Francisco, CA, USA

§Current affiliation: TScan Therapeutics, Waltham, MA, USA

¶Current affiliation: BeiGene, Cambridge, MA, USA

\*\*Current affiliation: Atara Biotherapeutics, Inc., South San Francisco, CA, USA

††Current affiliation: Pfizer, Inflammation and Immunology, Cambridge, MA, USA

**Corresponding authors:**

Michael H. Shaw

Takeda Development Center Americas, Inc. (TDCA), 95 Hayden Avenue, Lexington, MA 02421, USA

Email: [michael.shaw2@takeda.com](mailto:michael.shaw2@takeda.com)

Phone: +1 (617) 761-6834

Masanori Okaniwa

Takeda Development Center Americas, Inc. (TDCA), 95 Hayden Avenue, Lexington, MA 02421, USA

Email: [Masanori.Okaniwa2@takeda.com](mailto:Masanori.Okaniwa2@takeda.com)

Phone: +1 (617) 444-1596

**Authors’ Disclosures**

YS, ECC, DM, VAA, SI, VK, AM, JS, MM, MY, JH, LS, SH, VS, CG, ERR, HB, SM, TT, PS, JC, AOA-Y, CWA, MHS, MO disclose employment with Takeda. OG discloses previous employment with Takeda and current employment with Invicro, LLC. TDT discloses previous employment with Takeda and current employment with Atara Biotherapeutics, Inc.

**SUPPLEMENTAL TABLES**

**Supplementary Table 1. Cell line source, mycoplasma testing, and cell authentication**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cell Line** | **Source** | **Catalog Number** | **Mycoplasma Testing Date** | **Authentication\*** |
| THP1-Dual™ Human Acute Myeloid Leukemia Cells | Invivogen | thpd-nfis | 6/19/2017 | No |
| Human Embryonic Kidney 293 (HEK293T) Cells | ATCC | CRL-11268 | 2/15/2018 | No |
| ISRE-Nano Luc HEK293T Cells | Promega | CS190901† | 2/15/2018 | No |
| CT26.WT Cells | ATCC | CRL-2638 | 6/23/2016 | Yes |
| A20 Cells | ATCC | TIB-208 | 6/25/2015 | Yes |
| B16F10 Cells | ATCC | CRL-6475 | 7/22/2016 | Yes |

\*Cells were authenticated by IDEXX BioAnalytics CellCheck cell line authentication service with  
27-marker short tandem repeat strain analysis.

†Vector used at Promega to generate the cells.

**Supplementary Table 2.** Potency values of TAK-676 binding to mouse, rat, cynomolgus monkey, and human STING orthologs in TR-FRET-assay

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Assay** | **Value** | **Units** | **Standard Deviation** | **n** |
| mSTING TR-FRET IC50 | 0.010 | µM | 0.0008 | 3 |
| rSTING TR-FRET IC50 | 0.008 | µM | 0.001 | 3 |
| cSTING TR-FRET IC50 | 0.011 | µM | 0.001 | 3 |
| hSTING TR-FRET IC50 | 0.027 | µM | 0.008 | 20 |
| Values represent the arithmetic mean. All values were rounded. cSTING, cynomolgus monkey Stimulator of Interferon Genes; hSTING, human STING; IC50, concentration producing 50% inhibition; mSTING, mouse STING; rSTING, rat STING; SD, standard deviation; TRFRET, time-resolved fluorescence resonance energy transfer. | | | | |

**Supplementary Table 3.** Mean pharmacokinetic parameters in BALB/c mice bearing A20 tumors after intravenous administration of TAK-676 at 0.025, 0.125, 0.25, 0.5, and 2 mg/kg

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Matrix | Dosea (mg/kg) | tmax (h) | Cmax (nM) | Cmax/dose (nM)/(mg/kg) | AUC72 (nM\*h) | AUC72/dose (nM\*h)/(mg/kg) |
| Plasma | 0.025 | 0.083 | 38.3 | 1530 | 11.0 | 438 |
|  | 0.125 | 0.083 | 86.6 | 693 | 29.4 | 235 |
|  | 0.25 | 0.083 | 255 | 1020 | 76.9 | 307 |
|  | 0.5 | 0.083 | 587 | 1170 | 227 | 455 |
|  | 2 | 0.083 | 2250 | 1130 | 872 | 436 |
| Tumor | 0.025 | 0.17 | 10.9 | 436 | 204 | 8170 |
|  | 0.125 | 0.083 | 37.9 | 303 | 742 | 5940 |
|  | 0.25 | 0.083 | 65.5 | 262 | 399 | 1600 |
|  | 0.5 | 0.083 | 151 | 302 | 522 | 1040 |
|  | 2 | 0.083 | 342 | 171 | 1770 | 883 |

an=3 female BALB/c mice for each dose.

AUC72, area under the concentration-time curve from 0 to 72 hours; Cmax, maximum observed concentration; IV, intravenous; tmax, time to reach Cmax (in this case the plasma tmax was first sampling time after IV bolus).