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

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**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).