Supplements

Methods

Western blot
Proteins were extracted using a Triton-X100 buffer (150 mM sodium chloride, 50 mM Tris, 1% TritonX100, pH 7.4) containing proteinase inhibitors (Thermo Scientific). 20µg of protein were electrophoresed onto a 4–12% MES NuPAGE gel (Life Technologies) and transferred onto iBlot™ Transfer Stack, nitrocellulose (Invitrogen). Blots were incubated with specific antibodies agonist STING (Cell Signaling) or actine. Anti-rabbit-HRP was used as secondary antibody and BM Chemiluminescence Western Blotting Substrate (POD) (Sigma) was used for detection.

Viability assay
Cell viability and growth were evaluated using PrestoBlue® Cell Viability Reagent (Thermo-Fisher scientific) according to manufacturer protocol.

Generation of BL3750 STING knock out cell line
Sting KO BL3750 cell line was generated using the CRISPR Cas9 system.15 The plasmid containing the sequence of STING guide RNA was a gift from Dr Denise Monack (Stanford University).16 This plasmid was cotransfected with the px335 plasmid by electroporation using the AMAXA Nucleofector Technology. 48 hours after electroporation cells were cultured with STINGa to select for STING KO cells because the parental cell line is killed by STING. Clones were isolated by limiting dilution. Negative clones were submitted to a second limiting dilution. Negative clones, as assessed by western blotting, were used for experiments.

Table
Table S1: Flow cytometry panels

<table>
<thead>
<tr>
<th>Panel 1</th>
<th>Panel 2</th>
<th>Staining</th>
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<tbody>
<tr>
<td>Antibody</td>
<td>fluorophore</td>
<td>Antibody</td>
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<td>PE</td>
<td>anti-PDL-1</td>
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<td>PerCPCy5.5</td>
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<td>Ax700</td>
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<td>anti-Ki67</td>
<td>BV711</td>
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</tbody>
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Figures

A

[Images A20, B16F10, LLC1, EL4, H11, Pro B, CT26]

STING
Actin

B

A20

ADUs100
cGAMP
CpG

Fluorescence

µg/ml

0 0.002 0.02 0.2 2 20

C

BL3750

ADUs10C
cGAMP
CpG

Fluorescence

µg/ml

0 0.002 0.02 0.2 2 20

D

CT26

ADUs100
cGAMP
CpG

Fluorescence

µg/ml

0 0.002 0.02 0.2 2 20

E

B16F10

ADUs10C
cGAMP
CpG

Fluorescence

µg/ml

0 0.002 0.02 0.2 2 20

Figure S1 STING expression and sensitivity to STING agonist in various cancer cell lines.

A STING expression in A20, B16F10, LLC1, EL4, H11 and CT26 cancer cell lines as assessed by western blot analysis of whole protein extract. B-E, Viability assay using A20 lymphoma cells (B), BL3750 lymphoma cells (C), B16F10 melanoma cells (D) and CT26 colorectal cancer cells (E). Cells were incubated with increasing concentration of ADU-S100 (STINGα used in the rest of this study), GAMP (another STINGα) or CpG (TLR9 agonist). Viability was assessed after 24h incubation with the drugs using PrestoBlue. One experiment with triplicates. Statistical significance between control and treat groups was calculated using 2 way ANOVA. Error bars are SEM. Ns, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001.
Figure S2: STING agonist dose response at the local and distant tumor.

Mice were implanted with $5 \times 10^6$ A20 cells on both flanks. One tumor was used as injection site while the other was monitored for systemic effect. Mice received IT injection of STINGa in the right side tumor on days 6, 8 and 10 after tumor implantation. A, mice received 5ug, 25ug or 50ug of STINGa. B, mice received 50ug, 100ug or 200ug of STINGa. Error bars are SEM. Shown data are one experiment with 10 mice per group. Statistical significance of tumor growth was calculated 2 way ANOVA. C, Picture of the abdomen of tumor bearing mice one day after the last IT injection of indicated dose of STINGa.
Figure S3: Local or systemic delivery of the treatment and effect of the treatment in other tumor models.

Mice were implanted SC with tumor cells on both flanks. One tumor was used as injection site (A and C) while the other was monitored for systemic effect (B, D, E and F). **A-B** On days 6, 8 and 10 after A20 cells implantation, mice received IT injection of 5ug of STINGa with either IT or SC injection of 50ug of anti-GITR. **C-D** On days 6, 8, 10, 12, 14 and 16 after tumor implantation, mice received IT, SC or PT injection of 5ug of STINGa with SC injection of 50ug of anti-GITR. **E** 6, 8 and 10 days after 2F3 cells implantation, mice were treated with 20ug of the IT STINGa and 50ug of SC anti-GITR. **F** 6, 8 and 10 days after BL3750 implantation, mice were treated as in **E**. Error bars are SEM. Shown data are one experiment with 8-10 mice per group. Statistical significance of tumor growth was calculated 2 way ANOVA.
Figure S4: Local or systemic delivery of the treatment and effect of the treatment in other tumor models.

Cell population in the draining lymph node of treated tumor harvested as described in Figure 4A. T and B cell proportion one day post treatment (A) and one week post treatment (B). C, CD69+ cells among B cells (B220+). D, CD86+ cells among B cells (B220+).