Telomerase-Driven Telomeric DNA Modification in Cancer Cells Leads to Efficient Induction of cGAS-mediated Innate and Adaptive Immune Responses

Poster number: 697

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SUMMARY

- **The modified nucleotide 6-thio-2'-deoxyguanosine (THIO) induces telomerase-dependent telomeric DNA modification, DNA damage responses, and selective cancer cell death**
- **THIO-damaged telomeric fragments accumulate in cytosolic micronuclei that activate innate (cGAS/STING) and adaptive (T-cell) immune responses**
- **Sequential treatment with THIO followed by PD-(L)1 inhibitors results in profound and persistent tumor regression in advanced in vivo cancer models and induction of cancer type–specific immune memory**

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DISCLOSURES

- SMG, VV, MO are shareholders of MAIA Biotechnology, Inc.
- JWS is scientific co-founder, SAB member and shareholder of MAIA Biotechnology, Inc.
- IM, SS are not shareholders of MAIA Biotechnology, Inc.

INTRODUCTION

- Telomeres are linear repetitive sequences that protect the ends of the chromosomes from being recognized as double-strand breaks that would trigger the DNA damage response (DDR)[1]
- Telomerase, the enzyme responsible for extension of telomeres, is almost universally expressed in cancer cells, but not in most normal cells, making telomeres and telomerase attractive targets for highly specific anticancer therapy[2,3]
- The modified nucleoside 6-thio-2'-deoxyguanosine (6-thio-dG; THIO) is rapidly converted into the telomerase substrate 6-thio-2'-deoxyguanosine-5'-triphosphate (6-thio-dGTP) and incorporated into de novo synthesized telomeres
- We demonstrated that THIO targets cancer cell death through two key mechanisms[4-6] (Figure 1):
  - 1) formation of telomere dysfunction-induced foci (TIF), followed by rapid G2/M arrest or cell death of telomerase-positive cancer cells
  - 2) release of intracellular and extracellular THIO-containing telomeric DNA fragments that activate the cGAS-STING innate immune response pathway and initiate adaptive T-cell responses

Our aim was to characterize the mechanisms by which THIO targets cancer cells and evaluate the potential for sequential combination of THIO with anti–PD-(L)1 checkpoint inhibitors in advanced cancer

THIO targets cancer cells by activating DNA damage and immune responses

Figure 1

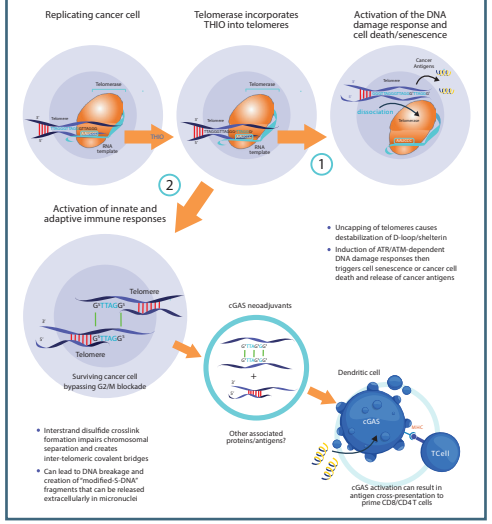

FIGURE 1: Mechanism of action of THIO. THIO targets replicating cancer cells by (1) initiation of DNA damage responses that lead to cell death/senescence and (2) cGAS/STING-dependent activation of innate and adaptive immune responses. Incorporation of THIO nucleotides into telomeres causes uncapping and disruption of the telomere-protecting shelterin complex. This disruption leads to telomere dysfunction-induced foci (TIF) formation, followed by rapid G2/M arrest or cell death of telomerase-positive cancer cells. In cells that bypass the G2/M checkpoint, THIO impairs chromosomal separation resulting in DNA damage and creation of THIO-modified S-DNA fragments. Release of these fragments activates innate and adaptive immune responses in a STING-dependent manner.

Abbreviations: cGAS, cyclic GMP–AMP synthase; MHC, major histocompatibility complex; STING, stimulator of interferon genes; THIO, 6-thio-2'-deoxyguanosine

THIO is highly effective and selective for cancer cells

- IC50 values ranged from 0.7 to 2.9 μM for the majority of cancer cell lines compared to ≥100 μM for normal cell lines; representative efficacy dose-response curves are shown in Figure 2

Figure 2

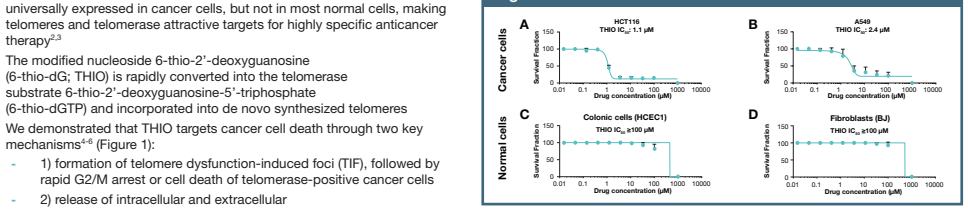

FIGURE 2: THIO selectively targets cancer cells. In vitro cell viability of (A,B) cancer cell lines (HCT116, A549) and (C,D) normal cell lines (HCEC1, BJ) treated with the indicated concentrations of 6-thio-dG every 3 days for 1 week using the CellTiter-Glo assay. All samples were analyzed in triplicate and SDs are from two independent experiments.

Abbreviations: THIO, 6-thio-2'-deoxyguanosine

THIO treatment leads to TIF formation in vitro

- THIO treatment induced TIFs in telomerase-positive cancer cells, but not in normal, telomerase-silent cells
- TIF formation is dependent on telomerase expression

Figure 3

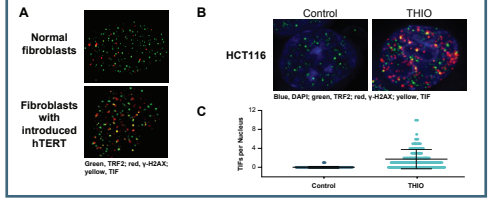

FIGURE 3: TIF formation after THIO treatment represented by colocalization of telomeres (green, TRF2) with DNA damage foci (red, γ-H2AX). A. TIF formation was dependent on telomerase (hTERT) expression in normal BJ fibroblasts treated with 10 μM THIO for 48 h. B. TIF formation in HCT116 cells treated with 3 μM THIO for 72 h. C. Quantification of TIFs per nucleus in HCT116 cells.

Abbreviations: H2AX, H2A.X variant histone; hTERT, human telomerase reverse transcriptase; THIO, 6-thio-2'-deoxyguanosine; TIF, telomere dysfunction-induced foci; TRF2, telomeric repeat binding factor 2

THIO induces DNA damage and formation of micronuclei

- Treatment of MC38 colorectal cancer (CRC) cells with THIO 1 μM for 48 h resulted in formation of telomeric cytosolic DNA and micronuclei
- Fused chromosomes were observed in THIO-treated cells, consistent with telomere damage

Figure 4

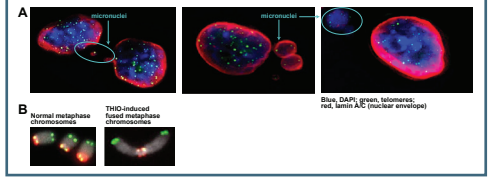

FIGURE 4: THIO-induced DNA damage and micronuclei formation. A. Representative images of two daughter cells in late telophase showing telomere signals and coated and uncoated micronuclei in MC38 CRC cells. Cells were treated with 1μM THIO for 48h and stained for telomeres (green, telomere probe) and nuclear envelope (red, lamin A/C) B. Representative images of normal metaphase chromosomes and fused metaphase chromosomes in THIO-treated cells.

Abbreviations: MC38, murine colon adenocarcinoma cells; THIO, 6-thio-2'-deoxyguanosine

THIO induces potent and self-complementary immune responses in CRC

- Immunophenotyping after THIO administration shows reduced MDSCs and increased NK and CD4/CD8 T-cell activation in the tumor microenvironment in vivo

Figure 5

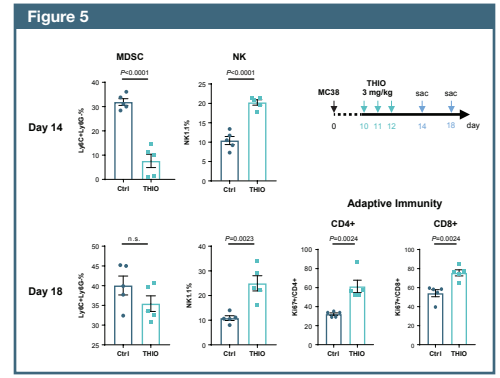

FIGURE 5: Immunophenotyping shows THIO-induced reduction of MDSCs and increase of T cell activation in the tumor microenvironment. C57BL/6 mice (n=5) were inoculated with 5x10[5] MC38 tumor cells and treated with THIO on days 10, 11, and 12. On days 14 and 18, tumor-infiltrating T cells were analyzed for the frequency of MDSCs (Ly6C+Ly6G-), NK cells (NK1+), CD4 T cells (K67 +/CD4+), and CD8 T cells (K67+/CD8+). Data are shown as mean ± SEM from 2 independent experiments, and *P*-values were determined by 2-tailed unpaired *t*-tests.

Abbreviations: CD, cluster of differentiation; MDSC, myeloid-derived suppressor cells; NK, natural killer cells; sac, sacrificed; THIO, 6-thio-2'-deoxyguanosine

THIO produces persistent tumor type–specific immune memory

- In a colorectal cancer (CRC) syngeneic murine model, sequential administration of THIO followed by an anti–PD-L1 therapy completely inhibited tumor growth
- Rechallenge of THIO-treated mice with 10 times more tumor cells resulted in spontaneous rejection of cells of the same tumor type without additional treatment

Figure 6

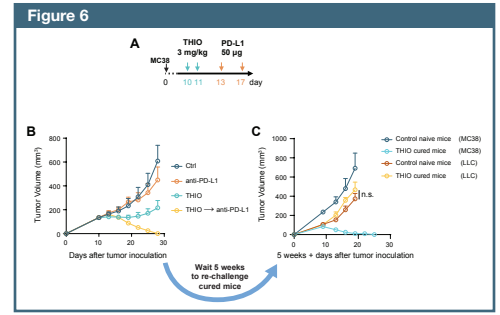

FIGURE 6: THIO overcomes PD-L1 blockade resistance and produces immune memory in advanced tumors. A, B. C57BL/6 mice (n = 5) were inoculated with 5x10[5] MC38 tumor cells and treated sequentially with THIO and/or an anti–PD-L1 antibody as shown in the timeline schema. Tumor volume was assessed at the indicated times. C. Five weeks later, tumor-free mice from THIO-treated group (n=5) and control naive mice (n=5) were re-challenged with 5x10[5] MC38 tumor cells on the opposite flank (left), and 5x10[5] LLC tumor cells were injected on the right flank. Data are shown as means ± SEM from two independent experiments, and *P*-value was determined by two-way ANOVA.

Abbreviations: LLC, lewis lung carcinoma; MC38, murine colon adenocarcinoma cells; PD-L1, programmed death-ligand 1; THIO, 6-thio-2'-deoxyguanosine

Sequential treatment with THIO enhances the efficacy of anti–PD-1 therapy in a humanized mouse model of SCLC

- Significant tumor regression was observed in mice treated sequentially with THIO followed by pembrolizumab compared to untreated and pembrolizumab only-treated mice.

Figure 7

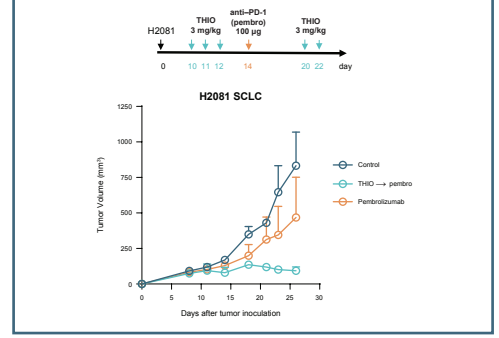

FIGURE 7: Sequential treatment with THIO followed by pembrolizumab reduces tumor burden in a humanized SCLC mice model. Mice were inoculated with 2x10[6] H2081 cells, and tumor-bearing mice were treated with pembrolizumab followed by THIO as shown in the schematic (n=5 per group). Tumor volume was assessed at the indicated times.

Abbreviations: PD-1, programmed death protein 1; SCLC, small cell lung cancer; THIO, 6-thio-2'-deoxyguanosine

Sequential treatment with THIO followed by cemiplimab is highly effective in an NSCLC model

- Compared with control group, both the cemiplimab and THIO groups showed an anti-tumor response
- Sequential treatment with THIO followed by cemiplimab induced a synergistic regression of LLC-derived tumors in NSCLC mice

Figure 8

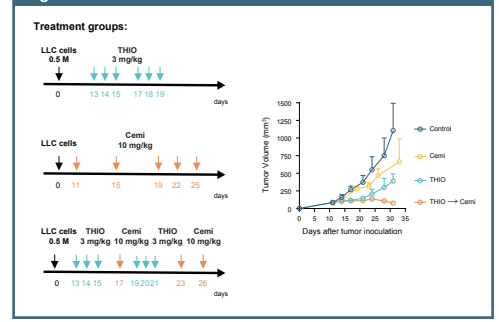

FIGURE 8: Sequential treatment with THIO followed by cemiplimab induces a synergistic response in NSCLC model. Mice were inoculated with 5x10[5] LLC cells, and tumor-bearing mice were treated with THIO and/or cemiplimab as shown (n=5 per group). Tumor volume was assessed at the indicated times.

Abbreviations: Cemi, cemiplimab; LLC, Lewis lung carcinoma; NSCLC, non-small cell lung cancer; THIO, 6-thio-2'-deoxyguanosine