New Dual-Pharmacophore Dinucleotide Prodrugs as Potent Telomere Targeting Anticancer Molecules

Ilgen Mender, Romina Girotti, Sergei Gryaznov

MAIA Biotechnology, Chicago, IL, USA

Abstract ENA24-0044

Introduction

- Telomerase is an enzyme that is expressed in more than 80% of all cancers, but not normal cells.[1]
- The cancer cell telomeres are attractive targets for development of specific anticancer drugs.
- The nucleoside analogue THIO (6-thio-2'-deoxyguanosine, a.k.a 6-thio-dG; 6SdG) is a first-in class telomerase-mediated telomere-targeting anticancer agent:
  - THIO is incorporated into de novo synthesized telomeres by telomerase, thus activating telomere damage responses, apoptotic pathways, and innate and adaptive immune responses.[2, 3, 4]
  - Currently, THIO is in Phase II clinical trials for the treatment of non-small cell lung cancer (NSCLC) patients resistant to immune checkpoint inhibitors.
- 5-Fluoro-2'deoxyuridine (5-FdU), a pyrimidine analogue, is a deoxyribonucleoside derivative of 5-fluorouracil (5-FU).
  - 5-FdU exerts its anticancer activity through thymidylate synthase enzyme via its active metabolites 5-fluoro- 2'deoxyuridine 5'-monophosphate (5FdUMP), which disrupts DNA metabolism.[5]
  - It was shown that the effects of 5-FdU on induction of telomere dysfunction, and reduction of POT1-TPP1 binding affinity to telomeres cause subsequent cell death via telomerase-dependent manner.[6]
- In this work we sought to target cancer cell telomeres using newly designed and synthesized divalent dinucleotides containing two telomere-modifying pharmacophores - 6SdG and/or 5-FdU nucleosides.
  - Dinucleotides offer a potential therapeutic approach due to their ability to provide two pharmacophores.
  - We demonstrate that 6SdG-containing prodrugs MAIA-2022-12 and MAIA-2021-20, show promising antitumor activity in various preclinical mouse models.

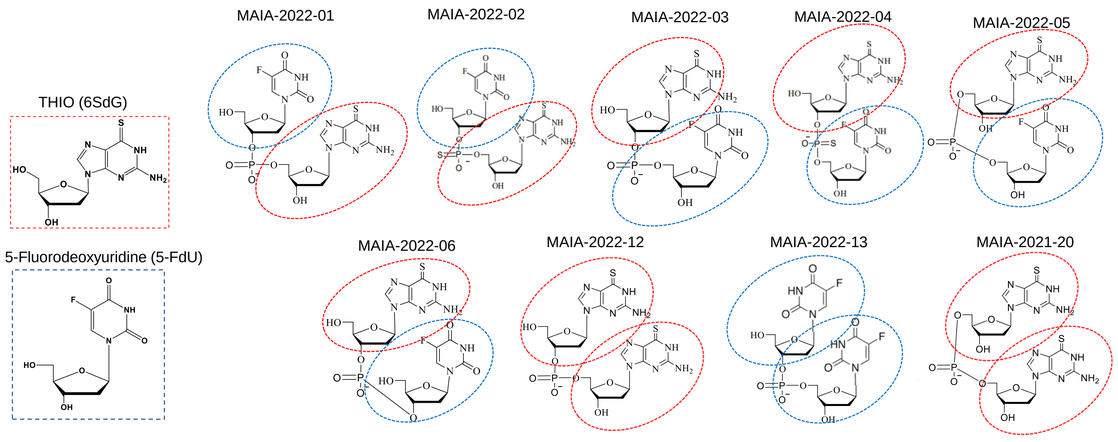
Methods

- Cell viability assays were used to determine the EC50s of the dinucleotides in various in vitro cell lines.
- A TIF (Telomere Dysfunction Induced Foci)-assay was used to determine the damage within telomeres induced by the tested compounds.
- In vivo syngeneic mouse models were used to evaluate the efficacy of the dinucleotides in combination with or without anti-PD-1 immune checkpoint-blocking antibodies.
- An in vivo syngeneic mouse model was used to evaluate the therapeutic effect of MAIA-2022-12 dinucleotide on $CD8^+$ T cells.

Results

- We designed a series of dinucleotides using combinations of THIO (6SdG pharmacophore) and 5-FdU components containing either linkage phosphorothioate (P=S) or linkage phosphodiester (P=O).

Figure 1. Chemical Structures of THIO, 5-FdU and newly designed divalent dinucleotides



- Stereoisomers of phosphorothioate-containing compounds (P=S) are more resistant to hydrolysis by nucleases than phosphodiester-containing counterparts (P=O).

Figure 2. Dinucleotides with phosphodiester bonds exhibit greater efficacy than the dinucleotides with phosphorothioate bonds

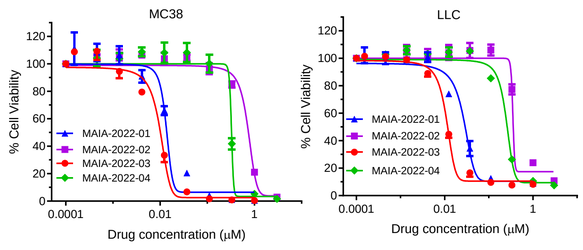


Table 1. EC50 (μM) values of dinucleotides in murine cancer cell lines

	LLC	MC38	Hep55-1C	SB28	HCC53N
MAIA-2022-03	0.01	0.01	0.01	0.01	0.04
MAIA-2022-01	0.03	0.01	0.03	0.03	0.08
MAIA-2022-04	0.25	0.33	0.25	0.31	0.79
MAIA-2022-02	0.35	0.74	0.61	0.34	2.42

- Mouse colon and lung cancer cell lines, MC38 and LLC, were more sensitive to pyrimidine analogue 5-FdU than purine analogue THIO (6SdG) in vitro.
- When molecules containing phosphodiester linkages (P=O) are cleaved, they form two pharmacophores, making them more potent than a single molecule of THIO alone.
- Our findings suggest that heterogenous compounds (6SdG-5-FdU or 5-FdU-6SdG) exhibit superior efficacy compared to homogenous compounds (6SdG-6SdG) in vitro.
- 5-FdU-5-FdU (MAIA-2022-13) demonstrate better efficacy than 6SdG (THIO) or 6SdG-6SdG (MAIA-2022-12/MAIA-2021-20) dinucleotides in vitro.

Table 2. EC50 values of dinucleotides in LLC and MC38 murine cancer cell lines

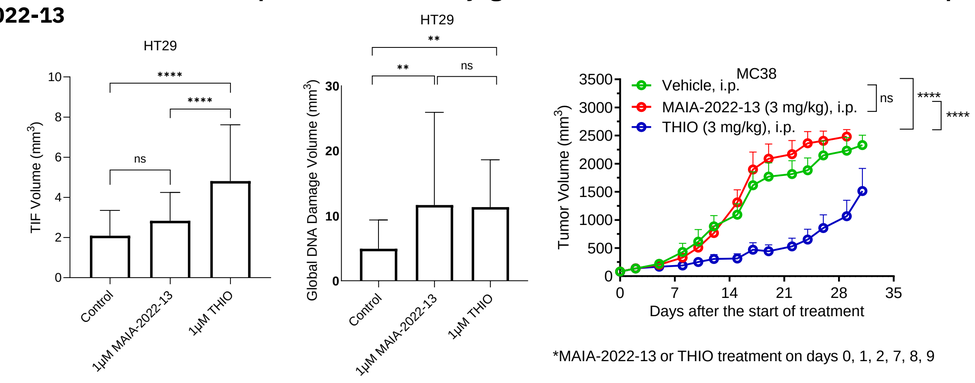
Compound Names	EC50 Values (μM) LLC
MAIA-2022-13	0.0004
5FdU	0.0006
MAIA-2022-05	0.01
MAIA-2022-03	0.01
MAIA-2022-01	0.02
MAIA-2022-06	0.02
MAIA-2022-12	0.20
MAIA-2021-20	0.75
THIO	1.07

Compound Names	EC50 Values (μM) MC38
MAIA-2022-13	0.002
5FdU	0.003
MAIA-2022-06	0.05
MAIA-2022-01	0.10
MAIA-2022-05	0.13
MAIA-2022-03	0.17
MAIA-2022-12	0.24
MAIA-2021-20	0.39
THIO	0.88

Results (continued)

- THIO induced TIFs and general DNA damage in HT29 cells. However, MAIA-2022-13 only induced general DNA damage, but not TIF formation, demonstrating its mechanism of action as a DNA damaging dinucleotide rather than damaging the telomeres.
- We found opposite effects in vivo with THIO and MAIA-2022-13 compared to in vitro. While THIO (3 mg/kg, i.p.) significantly delayed tumor growth in the MC38-derived syngeneic mouse model, MAIA-2022-13 (3 mg/kg, i.p.) did not show significant tumor growth delay compared to the vehicle control.

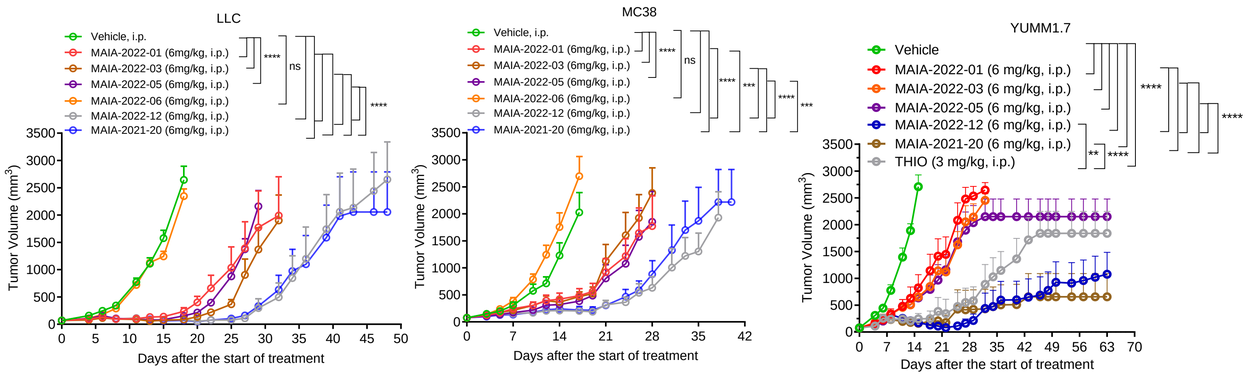
Figure 3. THIO Demonstrates a Superior Effect in the Syngeneic Mouse Model Derived from MC38 Compared to MAIA-2022-13



*MAIA-2022-13 or THIO treatment on days 0, 1, 2, 7, 8, 9

- MAIA-2022-06 did not inhibit tumor growth compared to control as it links 6SdG and 5-FdU nucleoside-based pharmacophores with 3', 3' – phosphodiester bond.
- MAIA-2022-12 and MAIA-2021-20 (homogenous compounds with 6SdG-6SdG components) demonstrated a superior efficacy compared to heterogenous compounds (MAIA-2022-01, MAIA-2022-03, MAIA-2022-05).
- These results indicate that the in vivo metabolic functions of MAIA-2022-12 and MAIA-2022-20 in various organs play a crucial role in the therapeutic effects of both MAIA-2022-12 and MAIA-2021-20 with 6SdG nucleoside-based pharmacophores.

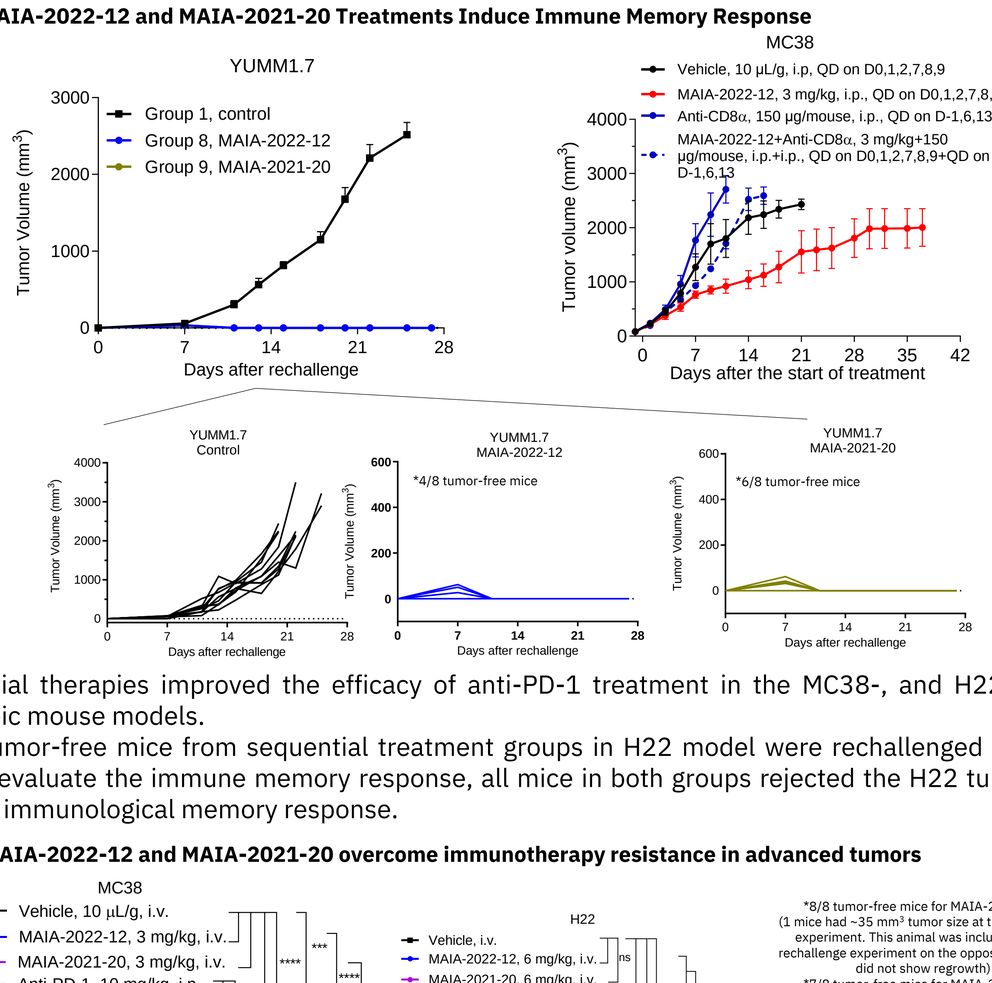
Figure 4. MAIA-2022-12 and MAIA-2021-20 Demonstrate a Superior Effect in Syngeneic Mouse Models



*MAIA-2022-01, MAIA-2022-03, MAIA-2022-05, MAIA-2022-06, MAIA-2022-12 or MAIA-2021-20 treatment on days 0, 1, 2, 7, 8, 9

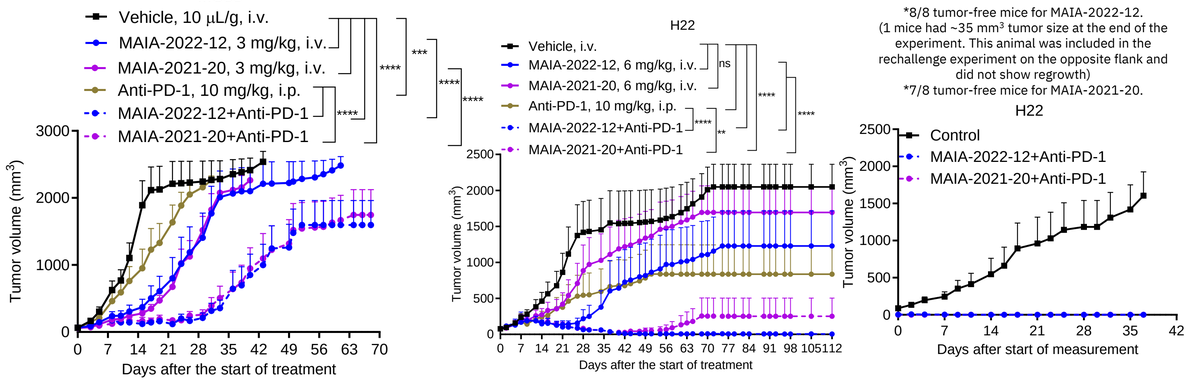
- The treatment of both MAIA-2022-12 and MAIA-2021-20 resulted in inhibition of tumor growth and showed immune memory against the same tumor type in YUMM1.7 model.
- The depletion of CD8+ T cells eliminated the therapeutic effect of MAIA-2022-12 in MC38 model, indicating the role of CD8 T cells in the antitumor effect of MAIA-2022-12.

Figure 5. MAIA-2022-12 and MAIA-2021-20 Treatments Induce Immune Memory Response



- Sequential therapies improved the efficacy of anti-PD-1 treatment in the MC38-, and H22-derived syngeneic mouse models.
- When tumor-free mice from sequential treatment groups in H22 model were rechallenged with H22 cells to evaluate the immune memory response, all mice in both groups rejected the H22 tumors and induced immunological memory response.

Figure 6. MAIA-2022-12 and MAIA-2021-20 overcome immunotherapy resistance in advanced tumors



*MAIA-2022-12 or MAIA-2021-20 treatment on days 0, 1, 2, 7, 8, 9 and anti-PD1 treatment on days 4, 12.

Conclusions

- **While dinucleotides that contain 5-FdU pharmacophores showed better efficacy in vitro compared to 6SdG-containing compounds, 6SdG-containing compounds (MAIA-2022-12 and MAIA-2021-20) showed higher efficacy than 5-FdU-containing compounds in vivo due to the metabolic functions of these dinucleotides.**
- **The treatment of both MAIA-2022-12 and MAIA-2021-20 resulted in tumor growth inhibition in preclinical models of lung cancer, colorectal carcinoma, melanoma and hepatocellular carcinoma and showed immune memory against same tumor type. Mice showed good tolerance to both MAIA-2022-12 and MAIA-2021-20.**
- **MAIA-2022-12 and MAIA-2021-20 overcome immunotherapy resistance in advanced tumors.**

References

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Presenting author contact

Ilgen Mender, Ph.D. (e-mail: imender@maiabiotech.com)

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