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Anti-Metastatic Activity of ISTH0047 – a Potent and Selective TGF-β2 Antisense Oligonucleotide - in Syngeneic Lung Metastatic Model of Mouse 4T1 Mammary Carcinoma

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Abstract

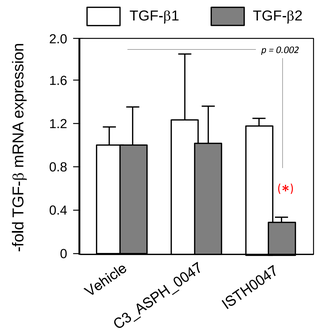
Transforming growth factor beta (TGF-β) isoforms are the primary mediators for TGF-β signaling *via* TGF-β receptors and downstream phosphorylation/dephosphorylation cascade. TGF-β is associated with a wide range of biological processes in Oncology, including tumor cell invasion, migration, angiogenesis, immunosuppression, as well as regulation of tumor stem cell properties. Mouse 4T1 mammary carcinoma cell line is a transplantable tumor cell line that is highly tumorigenic and invasive and, unlike most tumor models, can spontaneously metastasize from the primary tumor in the mammary gland to multiple distant sites including lymph nodes, blood, liver, lung, brain, and bone. Considering rather challenging preclinical evaluation of antitumor activity in tumor models, mouse 4T1 mammary carcinoma model has been widely used in the literature for evaluation of TGF-β antagonists. In this report, we describe the efficacy of ISTH0047 – a potent and selective TGF-β2 antisense oligonucleotide – in murine 4T1 primary tumors and lung metastasis following tumor cell injection into the mammary fat pad (orthotopic tumor model) of syngeneic Balb/c mice. Consistent with literature data generated with other classes of TGF-β antagonists (e.g., small-molecule kinase inhibitors, antibodies or TGF-β trap agents), although limited antitumor activity was demonstrated on primary tumor growth, marked and statistically significant decrease of lung metastasis number was observed upon subcutaneous administrations of ISTH0047. In addition, side by side comparison with murine surrogates of CTLA-4 or PD-1 antibodies indicated similar efficacy of all test items on lung metastasis in this model. Taken together, these encouraging results pave the way for in-depth preclinical evaluation of both *'seed and soil'* theory and efficacy of combination regimen (immunomodulation) for better clinical outcome.

Compound

ISTH0047 is a fully phosphorothioate-modified 17-mer oligodeoxynucleotide (ODN) with a '4+4' **LNA***-gapmer, and represents a potent and selective inhibitor of TGF-β2 protein expression *via* robust downregulation of target mRNA.

5'- **CAAA**GTATTTGGT**CTCC** *-3'*

ISTH0047-induced downregulation of TGF-β2

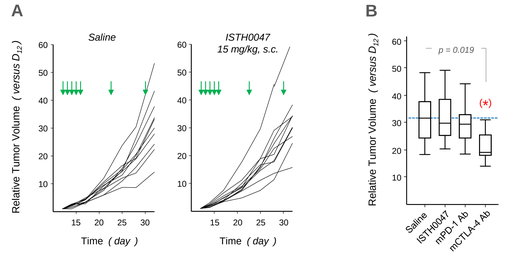


Mouse 4T1 breast cancer cells were incubated with vehicle (saline) or 3 µM of either a control (scrambled) ODN C3_ASPH_0047 or ISTH0047 in the absence of transfection reagent (gymnotic delivery). TGF-β1 and -β2 mRNA levels were analyzed after 96 h using qRT-PCR, and were normalized to corresponding HPRT1 mRNA levels. Results are expressed as the -fold TGF-β mRNA expression compared to vehicle treated cells, and represent mean ± SD values of 2 independent determinations performed in triplicates. Student's t-Test was performed to determine statistical significance.

Results: Upon gymnotic delivery to mouse 4T1 cells, ISTH0047 significantly and selectively downregulated the expression of TGF-β2 mRNA in a sequence-dependent fashion

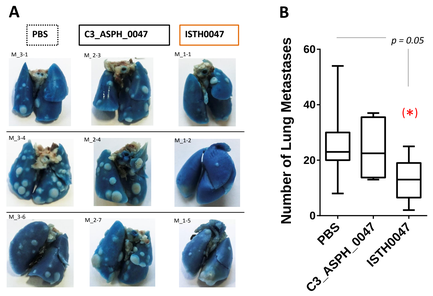
Effect of ISTH0047 on mouse 4T1 primary tumor growth

Mouse 4T1 breast cancer cells (10^4 cells) were orthotopically inoculated on Day 0 into the right lower mammary fat pad of Balb/c mice (n=10). On Day 12, when tumors reached a mean size of ~50 mm^3, mice were randomized and treatment was started. Mice were subcutaneously treated with 0.9% NaCl (saline) or 15 mg/kg ISTH0047 (green arrows indicate treatment days); or intraperitoneally treated with 10 mg/kg of either anti-mouse PD-1 antibody or anti-mouse CTLA-4 antibody (Q4Dx3). Animals were sacrificed on Day 32. ***(A)*** *Time-dependent evolution of primary tumor size (expressed as relative tumor volume compared to treatment start) in vehicle- and ISTH0047- treated mice.* ***(B)*** *The relative tumor volumes measured on Day 32 and displayed as box plots. Statistical analysis was performed using the non-parametric 2-independent samples Mann-Whitney-Wilcoxon Test.*



Results: Systemic administrations of either ISTH0047 or anti-mouse PD-1 antibody to 4T1 tumor-bearing mice did not induce any significant effect on primary tumor growth. Only the anti-mouse CTLA-4 antibody inhibited the primary tumor growth in this experiment.

Effect of ISTH0047 on lung metastasis from mouse 4T1 tumors



Mouse 4T1 breast cancer cells (10^5 cells) were orthotopically inoculated on Day 0 into the mammary fat pad of Balb/c mice (n=10). Mice were subcutaneously treated with PBS or with 20 mg/kg of the indicated ODNs (QDx5, then QW). Animals were sacrificed on Day 28 for determination of the number of lung metastases. PBS-treated mice started to severely suffer (weight loss) at Day 26 (data not shown). Statistical analysis was performed using non-parametric 2-independent samples Mann-Whitney-Wilcoxon Test.

(A) *Representative pictures of lung metastasis* ***(B)*** *Number of lung metastases as counted on Day 28. Results are represented as box plots.*

Result: Systemic administrations of ISTH0047 to 4T1 tumor-bearing mice resulted in a sequence-specific and significant decrease in the number of lung metastases from orthotopic 4T1 tumors.

Cells and Materials

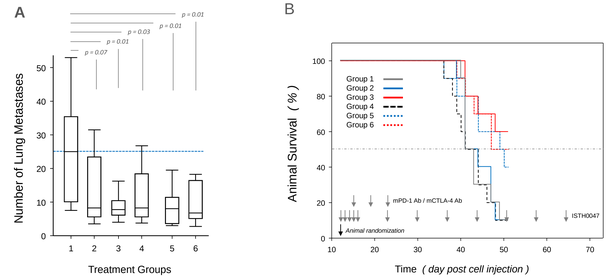
The murine 4T1 mammary carcinoma cells derive from transplantable tumor cell line that is highly tumorigenic and invasive in the immuno-competent mouse and which can spontaneously metastasize from the primary tumor mammary fat pad to multiple distant sites (e.g., lung).

ISTH0047 and C3_ASPH_0047 (synthesized by Biospring. Germany) are provided as lyophilized white powder. ODNs are dissolved and further diluted at appropriate concentrations in PBS or sterile saline.

Anti-mouse PD-1 (mPD-1) Ab (Rat IgG2a; clone RPM1-14) and anti-mouse CTLA-4 (mCTLA-4) Ab (Syrian Hamster IgG2; clone 9H10) were obtained from Bioxcell, USA.

Effect of ISTH0047 on number of lung metastases from mouse 4T1 tumors, and animal survival

Mouse 4T1 breast cancer cells (10^4 cells) were orthotopically inoculated on Day 0 into the right lower mammary fat pad of Balb/c mice (n=10). ***(A)*** *On Day 12, when tumors reached a mean size of ~50 mm^3, mice were randomized and the subcutaneous treatment (QDx5, then QW) with saline or 15 mg/kg ISTH0047 was started. The intraperitoneal treatment (Q4Dx3) with 10 mg/kg of either anti-mouse PD-1 or anti-mouse CTLA-4 antibody started on Day 15. Animals were sacrificed on Day 32 for determination of lung metastasis. Number of lung metastases are represented as box plots. Statistical analysis was performed using non-parametric 2-independent samples Mann-Whitney-Wilcoxon Test* ***(B)*** *Animal survival (beyond Day 32) was evaluated in a separate study using similar treatments and study design (except that primary tumors were surgically resected when reaching ~300-mm^3 size). Results are represented in a classical Kaplan-Meyer graph format.*



1. Vehicle (saline)
2. mPD-1 Ab (10 mg/kg, i.p., Q4Dx3)
3. mCTLA-4 Ab (10 mg/kg, i.p., Q4Dx3)
4. ISTH0047 (15 mg/kg, s.c., QDx5, then QW)
5. ISTH0047 + mPD-1 Ab
6. ISTH0047 + mCTLA-4 Ab

Result: Similar pronounced (70%) and marked or statistically significant ($p<0.05$) decrease in number of lung metastases in the test item-treated groups. When applied in combination regimen, no additivity between ISTH0047 and either anti-mouse PD-1 or anti-mouse CTLA-4 antibodies was detected. A survival benefit was observed for mice treated with the anti-mouse CTLA-4 antibody, and the combination of ISTH0047 with either anti-mouse PD-1 or anti-mouse CTLA-4 antibodies.

Conclusions

1. Upon gymnotic delivery to murine 4T1 breast cancer cells, ISTH0047 potently and specifically suppressed (target) TGF-β2 mRNA
2. Upon systemic administrations to orthotopic 4T1 tumor-bearing mice, ISTH0047 significantly decreased the number of lung metastasis, whereas no effect on the primary tumor growth was observed
3. The anti-metastatic potency of ISTH0047 on lung metastasis in this model was similar to that of anti-mouse PD-1 or anti-mouse CTLA-4 antibodies
4. Combination of ISTH0047 with anti-mouse PD-1 or anti-mouse CTLA-4 antibodies did not result in additivity or synergy on lung metastases
5. Effect of the anti-mouse CTLA-4 antibody, and the combination of ISTH0047 and either anti-mouse PD-1 or anti-mouse CTLA-4 antibodies resulted in a survival benefit
6. The use of ISTH0047, targeting TGF-β2 mRNA, is an attractive therapeutic approach to inhibit breast cancer metastasis, for instance. However, further preclinical profiling is needed to warrant clinical investigation

** Use of LNA-modified gapmers is performed under a license from Roche (formerly Santaris Pharma).*

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