# Mouse Renca Renal Cell Carcinoma Syngeneic Model to Evaluate Efficacy of Novel Antisense Oligonucleotides Targeting Transforming Growth Factor beta (TGF-β) Isoforms

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**Abstract # 3126**

**Background** : Transforming Growth Factor beta (TGF-β) represents a family of cytokines, which function as the primary mediators for TGF-β signaling *via* TGF-β receptor type II (TβRII) and both non-canonical and canonical downstream signaling pathways. 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. Hence, optimal preclinical evaluation of efficacy of TGF-β antagonists is challenging. Isarna Therapeutics has designed and developed selective and potent LNA-modified antisense oligonucleotides targeting the various TGF-β isoforms. In order to adequately evaluate selected preclinical development candidates, Oncodesign has developed customized experimental mouse Renca renal cell carcinoma models in syngeneic and/or immunodeficient mice. The Renca cell line was established from a murine transplantable renal adenocarcinoma of spontaneous origin, and has been used under various experimental conditions: (1) subcutaneous tumor model by inoculating cells into the flanks of the animals; (2) the pulmonary metastatic tumor model by an intravenous injection of cells into the tail vein; and (3) the orthotopic tumor model by injecting cells into the renal subcapsule (and subsequent pulmonary metastasis). Outcome of this development program and preliminary results for selected TGF-β antisense oligonucleotides are presented and discussed.

**Figure 1 : The role of TGF-β in tumor development (multi-modal tumor-promoting effects)**

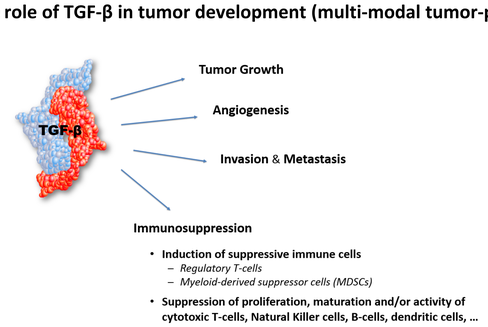
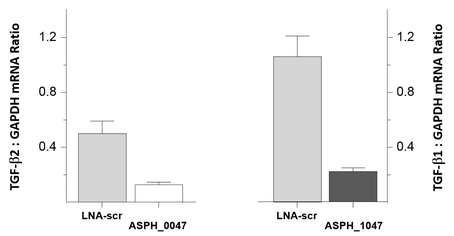


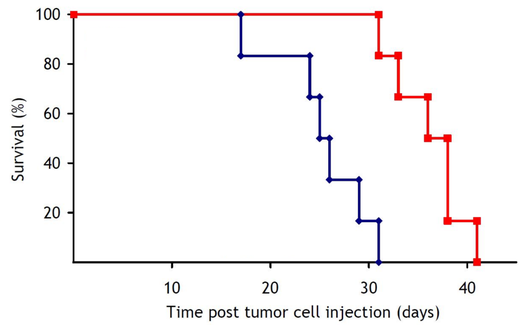

**Figure 2 : TGF-β1 and TGF-β2 mRNA expression following gymnotic delivery of selected TGF-β oligonucleotides constructs in mouse Renca RCC cell-based assays**




*Experimental design: Mouse Renca cells were incubated with 3 µM of either **ASPH_0047** (selective TGF-β2 antisense oligonucleotide; white bar), **ASPH_1047** (selective TGF-β1 antisense oligonucleotide; dark bar), or **LNA-scr** (scrambled oligonucleotide; grey bars) for 72 hours in the absence of any transfecting reagent (gymnotic delivery). Cell extracts were then processed, and mRNA expression levels determined by bDNA assay.*

**Results :** Confirmed potent target mRNA downregulation (70-80 %) in mouse Renca cells after gymnotic delivery of either ASPH_0047 (TGF-β2) or ASPH_1047 (TGF-β1).

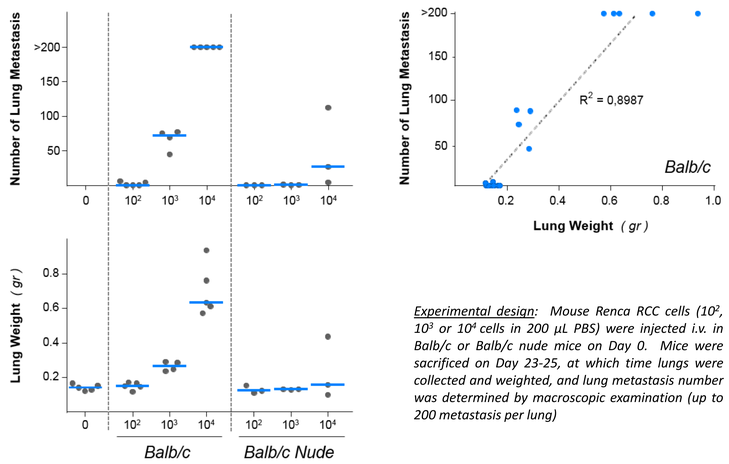
**Figure 3 : Survival of Balb/c mice bearing orthotopic (kidney) mouse Renca RCC tumors**




*Experimental design: Mouse Renca RCC cells were injected into the renal subcapsule (othotopic implantation) of Balb/c mice on Day 0. Mice were randomized (based on body weight) on Day 4, and treated (QD, p.o.) with either vehicle ( ◆ ) or sorafenib at 100 mg/kg ( ■ ) for three consecutive weeks.*

**Results :** Orthotopic mouse Renca renal cell carcinoma model remains responsive to standard of care treatment (i.e., sorafenib), as demonstrated by survival benefit of about 10 days in comparison to vehicle-treated mice.

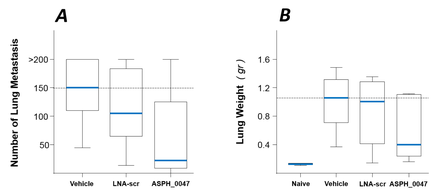
**Figure 4 : Development of lung metastasis model (i.v. administration of Renca cells) in Balb/c and Balb/c nude mice**




*Experimental design: Mouse Renca RCC cells ($10^2$, $10^3$ or $10^4$ cells in 200 µL PBS) were injected i.v. in Balb/c or Balb/c nude mice on Day 0. Mice were sacrificed on Day 23-25, at which time lungs were collected and weighted, and lung metastasis number was determined by macroscopic examination (up to 200 metastasis per lung)*

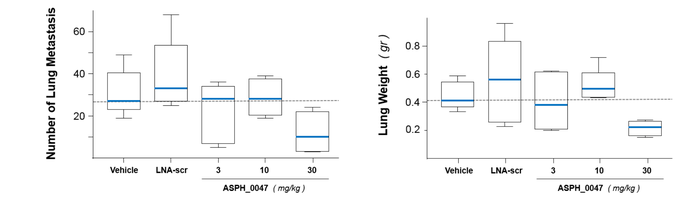
**Results :** In Balb/c mice, i.v. injection of mouse Renca RCC cells led to cell number-dependent increase in macroscopic lung metastasis (with median metastasis number of 72 and more than 200, when $10^3$ and $10^4$ cells were injected, respectively). In Balb/c nude mice, similar i.v. injection of increasing Renca RCC cells led to development of significant amount of lung metastases only when $10^4$ cells were injected (median number of metastases of 27). Extent of lung metastasis was also 'predicted' by lung weight determination, as we observed a good correlation between individual lung weights and metastasis numbers ($R^2$ = 0,8987)

**Figure 5 : Effect of systemic treatment of Balb/c mice with ASPH_0047 (selective TGF-β2 antisense oligonucleotide) on lung metastasis in orthotopic (kidney) mouse Renca RCC model**




*Experimental design: Balb/c mice were injected with mouse Renca cells into renal subcapsule on Day 0. Systemic treatment with vehicle or indicated oligonucleotides started on Day 7 (**A**; 50 mg/kg, s.c., twice weekly), or on Day 1 (**B**; 12.5 mg/kg, s.c., twice weekly) for two consecutive weeks. Number of lung metastasis was macroscopically evaluated, and level of lung metastasis was determined by either number of metastasis (**A**) or based on lung weight (**B**).*

**Figure 6 : Effect of systemic treatment of Balb/c mice with ASPH_0047 (selective TGF-β2 antisense oligonucleotide) on lung metastasis in i.v. mouse Renca RCC model**




*Experimental design: Balb/c mice were injected i.v. with mouse Renca cells on Day 0. Systemic treatment with vehicle or indicated oligonucleotides started on Day 7, and continued for 26-27 days (s.c., twice weekly at indicated doses). The number of metastases was counted (left panel) and lung weights measured (right panel).*

**Results :** Under described experimental designs, Balb/c mice treated with ASPH_0047 showed reduced number of lung metastases or reduced lung weight (lung weight correlates with extent of lung metastasis) in mouse Renca RCC lung metastasis models. In both studies, results are represented as box plot; with median values, upper and lower quartiles, and 90th and 10th percentiles)

**Conclusions :**

1. Marked downregulation of TGF-β2 and TGF-β1 mRNA after gymnotic delivery of ASPH_0047 and ASPH_1047, respectively, in mouse Renca RCC cell-based assays
2. Syngeneic Balb/c mice developed significant number of lung metastases when mouse Renca RCC cells were injected i.v., and to a lesser extent in Balb/c nude mice.
3. Confirmed trend in reduction of lung metastasis number (and consequently lung weight) in syngeneic Balb/c mice bearing orthotopic (kidney) mouse Renca RCC tumors and mice injected i.v. with Renca cells following systemic treatment with ASPH_0047, and not control scrambled oligonucleotide.
4. Administration route and/or treatment schedule for ASPH_0047 may require some further optimization (e.g., based on tissue PK data) to increase inter-studies reproducibility and consistency.
5. Fast-growing tumors in syngeneic models make it difficult to study potential immune-related effects of selected oligonucleotides as treatment window spans only 2-3 weeks.

*a. The authors wish to acknowledge Axolabs (Kulmbach, Germany) for the quality of their technical contribution in the presented studies.*

*b. Use of LNA-modified gapmers is performed under a license from Santaris Pharma.*

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