# Development of Antisense H-Ras Oligomers and Investigation of Their Potential as a New Drug Against Liver Cancer: In Vitro and In Vivo Studies

**Synopsis**

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According to the World Cancer Report 2020, cancer was one of the major causes of mortality globally in 2020, with an anticipated 10 million deaths. According to the National Cancer Institute (NCI), USA, cancer kills more men than women and is sex biased. Before the age of 70, the mortality rate from cancer was 189.5 per 100,000 male participants compared to 135.7 per 100,000 female participants. In most countries, cancer is the main cause of death (WHO, 2019). Hepatocellular carcinoma (HCC), a disease that has a high death rate and contributes significantly to cancer-related fatalities worldwide, is usually diagnosed when it has progressed to a point when chemotherapy is the only treatment option. The majority of anticancer drugs are very cytotoxic. The majority of chemotherapeutic substitutes are far from meeting expectations. However, genetic treatments like antisense oligomer (ASO) showed remarkable HCC inhibitory effectiveness without appreciable harm when contrasted with conventional chemotherapeutic alternatives. In order to compare *H-Ras* targeted genetic therapy with a commercial docetaxel formulation (DXT), we first assessed how well ASO treatment against mutant *H-Ras* gene suppressed HCC in rats. After this significant finding, we used intravenous administration of the corresponding ASO in chemically induced HCC rats to inhibit mutated H-Ras or c-raf.1 gene expressions often linked to HCC development and progression. Mutations in the Ras-Raf-MAP (mitogen-activated protein) kinase pathway, specifically the *H-Ras* mutation (located in codon 61 at position 12) and the *Raf-1 (c-raf.1)* mutation (at CRAFP261A), have long been associated with the development and progression of HCC. Further effort was made to ascertain the efficacy and mechanistic intervention of the treatments. This study examined how well *H-Ras*-targeted gene therapy and taxotere®, a commercially available version of docetaxel, prevented carcinogen-induced HCC in rats.

Numerous biochemical, histological, pharmacokinetic, histochemical, and morphological features were analyzed in combination with confocal and electron microscopic investigations in order to reach the conclusion. The pharmacokinetic profile, in vitro hemolysis, in vivo hepatic uptake, tissue distribution in specific highly perfused organs, effect in normal rats, and antineoplastic efficacy in carcinogen-induced HCC in rats were all assessed. Additionally, the physicochemical characterization of PS-ASO against the mutated H-*Ras* gene was completed.

In rats with HCC, these outcomes were contrasted with DXT treatment. In addition, in situ hybridization of mutated H-Ras expression was carried out in the experimental rats to assess the expression patterns of Hep-par-I, CK-7, CD-15, and p53 by immunohistochemical techniques, scanning electron microscopy evaluation of hepatic architecture, various hepatic marker enzyme levels, and caspase-3/9 apoptotic enzyme activities. Next, using the appropriate ASOs to block H-Ras or c-raf.1 gene expression, we looked at both in vitro and in vivo studies.

Compared to DTX, PS-ASO showed little in vitro hemolysis (<3%), a time-dependent hepatic absorption, and a longer blood residence time in vivo. It showed no negative signs in healthy rats. Even though PS-ASO was initially distributed less widely in the lung than in the liver and kidney, after eight hours, it accumulated more in the lung than the kidney. When given to rats for six weeks, PS-ASO (as opposed to DTX) showed better anticancer potential in avoiding chemically-induced carcinogenesis by inhibiting H-ras gene expression, causing caspase-3/9-mediated apoptosis, and reducing specific immonohistochemical modulations. It prevented lung metastases from HCC in the experimental animals.

Nanosize and well-dissociated ASOs tested against mutated *H-Ras* and *c-raf.1* in human HCC cells showed a range of IC50 values, while normal human liver cells showed no discernible damage at the experimental dose range. There was an even distribution of tagged ASOs in a healthy rat liver. After being exposed to ASOs, HepG2 and Huh7 cells showed signs of apoptosis and an increase in mitochondrial membrane potential. ASOs reduced the number of localized liver lesions and tumors by a large margin. ASOs altered the quantity of localized liver lesions and dramatically decreased the incidence of tumors. ASO treatments increased p53 expression, suppressed the production of the relevant genes, and reduced the expression of Hep Par I and HSP70 proteins in rat livers with HCC. ASO therapies altered the amounts of the proteins caspase-3 and -9 in HCC rats. When ASO was given to normal rats, hepatic marker enzyme levels remained unchanged, but they improved toward normal levels in rats treated with carcinogens.

PS-ASO (or ASO) was continuously and continuously released from the blood, allowing it to penetrate the liver tissues of the experimental rat. Additionally, ASOs were dispersed well throughout the lung and kidney tissues. For healthy animals, it had no adverse effects. In inhibiting the formation of chemically created tumors in rats, PS-ASO treatment (for 6 weeks) showed noticeably better therapeutic potential than commercially available docetaxel. Because

these two caspases were investigated in the current study, PS-ASOs demonstrated therapeutic promise by increasing at least caspase-3 and caspase-9 activity. PS-ASO also reduced the chance that the original HCC would develop into a lung tumor that spread to other places.

The presence of mutant Ras and c-raf.1 genes was shown by the HCC model developed here using the in-situ hybridization technique. Treating the mutant Ras or Raf gene with antisense oligonucleotides (PS-ASOs, sometimes referred to as ASOs here) had distinct effects on apoptosis induction. Changes in mitochondrial membrane depolarization as well as a range of cellular histochemical and biochemical markers were among these impacts. In particular, *c-raf.1* gene inhibition was less effective than mutant *Ras* gene suppression generated by ASO in reducing HCC. According to this study, PS-ASO may be able to target the mutant *H-Ras* gene and affect other proteins associated with the development of HCC more generally.

PS-ASO treatment, which targets the mutated *H-Ras* gene, holds a lot of promise for treating HCC in the future and may also be utilized to treat other malignancies. Genetic therapy using PS-ASO was shown to be far more effective than DXT at preventing HCC in rats. The therapeutic effectiveness of ASO treatment against mutant *H-Ras* was higher than that of ASO treatment against *c-raf.1*. Consequently, blocking mutant *H-Ras* instead of *c-raf.1* may significantly affect the prevention of HCC in rats. It is necessary to conduct more research.

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