Published in final edited form as:

Pancreatology. 2015 July ; 15(0): S39–S43. doi:10.1016/j.pan.2015.05.472.

Minnelide, a novel drug for pancreatic and liver cancer

Sulagna Banerjee¹ and Ashok Saluja^{1,2,*}

¹Division of Basic and Translational Research, Dept. of Surgery, University of Minnesota, Minneapolis, MN, 55455, USA

²Masonic Cancer Center, University of Minnesota, Minneapolis, MN, 55455, USA

Abstract

Pancreatic cancer is the 10th leading cause of all new cancer cases for men and the fourth leading cause of death across genders, having very poor prognosis and survival rates. The current standard of care Gemcitabine fails to add any survival benefit for this disease (www.cancer.gov). Though the incidence of pancreatic cancer is found to be higher in developed countries, the aggressive biology of the cancer, its high rate of recurrence and chemo-resistance make it a formidable disease in all parts of the globe. Hepatocellular carcinoma (HCC) or liver cancer, on the other hand affects almost 750,000 people world wide with 84% of the cases coming from underdeveloped or developing countries. Our studies show that Minnelide, a water soluble pro-drug of triptolide (active compound from a chinese herb) is very effective against a number of malignant diseases. The current study discusses the efficacy of this compound in pancreatic and liver cancer.

Introduction

Cancer has a severe impact on individuals and communities. Not only does it lead to disability and death, its treatment costs and associated loss of income can quickly undermine family finances. The most commonly diagnosed hepatobiliary cancers across the globe are hepatocellular carcinoma (HCC) and pancreatic cancer. Pancreatic cancer affects close to 250000 people worldwide [1]. The incidence of pancreatic cancer correlates with increasing age with a peak incidence of the disease occurring in the 65–75 year age group [2]. Untreated metastatic pancreatic cancer has a median survival of 3–5 months and 6–10 months for locally advanced disease [3]. The majority of cases are diagnosed in the advanced stages, making curative therapy impossible and leading to poor prognosis and incidence equaling mortality [4]. Though the incidence of pancreatic cancer is found to be higher in developed countries, the aggressive biology of the cancer, its high rate of

*Corresponding Author: Ashok K Saluja, Division for Basic and Translational Research, Dept. Of Surgery, University of Minnesota, 515 Delaware Street SE, Minneapolis, MN, 55455, USA, asaluja@umn.edu, Phone: 612 624 8108, Fax: 612 624 8909.

Conflict of interest

University of Minnesota has filed a patent for Minnelide, which has been licensed to Minneamrita Therapeutics, LLC. AKS has financial interests in this company. SB is a consultant with Minneamrita Therapeutics.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

recurrence and chemo-resistance make it a formidable disease in all parts of the globe. Hepatocellular carcinoma (HCC) or liver cancer, on the other hand affects almost 750,000 people world wide with 84% of the cases coming from underdeveloped or developing countries. Worldwide, HCC is the fifth most common of all malignancies and causes approximately one million deaths annually [5].

This review is focused on efficacy of a novel chemotherapeutic compound, Minnelide, that has shown promise in treatment of both pancreatic and liver cancers.

Etiology of pancreatic and hepatic cancers

The etiology of pancreatic adenocarcinoma remains poorly defined, although important clues of disease pathogenesis have emerged from epidemiological and genetic studies. Incidence of acute and chronic pancreatitis, gallstones and lifestyle choices like diet, smoking, alcohol consumptions are thought to be key factors for development of pancreatic cancer [6]. Cigarette smoking has the strongest overall association and thought to account for one-quarter of all patients diagnosed [7]. Another factor associated with increased risk of pancreatic adenocarcinoma include saturated fat intake, exposure to non-chlorinated solvents, and the pesticide dichlorodiphenyl trichloroethane (DDT), although the overall contribution of these is probably small [8].

Most cases of HCC (80%) arise in eastern Asia and sub-Saharan Africa, where the main risk factor is chronic infection with hepatitis B virus. However, in North America, Europe, and Japan, infection with hepatitis C virus is the dominant risk factor, together with alcohol use [9]. Both heavy alcohol use and chronic infection with Hepatitis B/C virus leads to liver cirrhosis that contributes largely to development of hepatocellular carcinoma. Other than these, smoking, obesity, inherited metabolic diseases have also been listed as potential pre-disposing factors for liver cancer.

Hepatic cancers can be primary or secondary. Primary hepatic cancer arises in the liver cells, particularly those with cirrhosis. Liver is also one of the seats for spread of cancers from other organs. Tumors from other regions of the GI tract (colorectal, gall bladder, stomach, pancreas) metastasize to the liver at an advanced stage.

Standard of Care in pancreatic and liver cancer

Over the past several decades, PDAC survival rates have not improved due in large part to delayed detection, metastasis [10], and drug resistance of PDAC cells [11]. This underscores the importance of effective, targeted chemotherapies to treat pancreatic cancer as it grows aggressively, is highly metastatic and yet remains resistant to chemotherapy. There are currently three main FDA-approved therapies. 5-fluorouracil, a pyrimidine analog, has been used since the 1970s. Gemcitabine, a nucleoside analog, was approved in 1996 and provides a 1.5-month survival advantage over 5-fluorouracil. Erlotinib, approved in 2006, adds a mere 10 days to the survival rate achieved by gemcitabine [12]. Erlotinib inhibits the tyrosine kinase epidermal growth factor receptor (EGFR), and its administration has shown modest benefit in clinical trials [13–15]. As of September 2013, Abraxene, an albumin binding form of paclitaxel, has been approved by FDA for pancreatic cancer, as it showed a

survival advantage of 5 months when used in combination with Gemcitabine. In spite of this, the need for more effective chemotherapeutic agents conferring better survival advantages is needed in this disease.

In case of hepatocellular carcinoma, disease is often asymptomatic, thus patients are commonly diagnosed at very advanced stages. This leads to a poor initial prognosis, with more than 50% of these patients dying within 1 year after their definitive diagnosis [16]. Systemic treatment in advanced metastatic cases is limited to sorafenib (Nexavar, Bayer HealthCare Pharmaceuticals-Onyx Pharmaceuticals), a broad-spectrum multi-kinase inhibitor. Sorafenib primarily inhibits the RAF-Erk-MAPK signaling in tumor cells, exerting an effect on cell viability as well as proliferation and angiogenesis [17]. Overall, sorafenib treatment results in a statistically significant, but clinically very modest, improvement in overall survival: a randomized, placebo-controlled double-blind phase III-study showed median survival increased from 7.9 to 10.7 months [18]. However, patient noncompliance is often an issue, with high drug costs and adverse side effects (most frequently diarrhea, fatigue, and hand-foot syndrome) [19, 20] commonly cited as reasons for its discontinuance.

In particularly aggressive cancers, such as advanced metastatic HCC, the complex nature of the cell-to-cell and cellular environment interactions is well recognized. Certainly it can be argued that treatment should entail a multi-drug regimen, particularly if the drugs act by different mechanisms of action, thereby allowing for a potentially improved effect. This is certainly true for hepatocellular carcinoma, where multiple studies of combination therapies are emerging [17, 21]. While some results have been encouraging, a standardized combination treatment for HCC has not yet been implemented. Sorafenib continues to have some utility in the treatment of the patient with advanced disease; however, adding an additional drug to the patient's treatment course may allow for an increased therapeutic benefit, while reducing the amount of either drug required, thereby decreasing potential adverse side effects.

Minnelide an effective therapy against pancreatic cancer

Triptolide, a diterpene triepoxide extracted from the Chinese plant *Trypterygium wilfordii*, has shown a lot of promise as a potential chemotherapeutic option in multiple tumor cell lines. Owing to its limited solubility *in vivo*, University of Minnesota synthesized Minnelide (Minne=Minnesota and -lide = triptolide) by adding a phosphate ester group that will generate a chemically unstable compound. Minnelide was found to be extremely effective in both pancreatic [22] and liver cancer [23].

Triptolide and Minnelide induce death to pancreatic cancer cell lines

Minnelide has been extensively tested *in vitro* against pancreatic cancer. *In vitro*, triptolide, the active drug of Minnelide, decreased cell viability in a number of pancreatic cancer cell lines (Figure 1A). Cell death was found to be apoptotic in some cell lines and autophagy induced in others [24].

Minnelide reduces tumor volume in multiple animal models

Minnelide reduced tumor volume in multiple models of pancreatic cancer. In both primary and metastatic cell line derived model, pancreatic cancer cell lines (Mia-PACA2, S2013 or AsPC1) were implanted orthotopically in the pancreas of the animal. Gross analysis of the control mice in all the three cell lines demonstrated the presence of ascites, multiple tumors and metastasis in all animals. Conversely, Minnelide-injected mice did not have ascites, and did not spread as much from the primary site of injection. Further analysis of the pancreatic tumors showed a significant decrease in tumor weight and volume in Minnelide treated animals when compared to the untreated ones. (Figure 1B, C).

In the animal model using the ascites derived ASPC1 cell line, the median survival of the control saline-treated group was 36 days, with very large tumors at time of sacrifice. Minnelide treated mice, on the other hand, possessed no palpable tumors on day 100 of treatment. Further, even on stopping treatment with Minnelide and following the animals till the end of experiment (385 days), the tumors did not recur or show any signs for toxicity (Figure 1D).

This experiment on different pancreatic cancer orthotopic models in mice demonstrated that Minnelide was effective in reducing tumor burden in pancreatic cancer cell line. Further, it also induced tumor regression, reduced metastasis and increased survival in these different mouse models.

Minnelide is effective against patient tumor derived xenografts

One of the limitations of a cell line derived models is the accumulation of mutations over time in culture conditions, making the genetically different from the original tumor. To address this concern, we tested its effect on human pancreatic cancer xenografts. Tumors regressed in Minnelide-treated animals and as seen in the orthotopic model, the tumors did not recur even when treatment was stopped (Figure 1E).

Minnelide, an effective combination against hepatocellular carcinoma

Sorafenib is an oral multi-kinase inhibitor with anti-proliferative activity in HCC, owing to its broad-based inhibition of cellular signaling and tumor angiogenesis. Sorafenib exerts its tumor cell anti-proliferative effects primarily by targeting Raf/mitogen-activated protein extracellular kinase/ERK signaling at the level of Raf kinase, while providing an antiangiogenic effect by targeting vascular endothelial growth factor (VEGF) receptor2/-3 and platelet-derived growth factor receptor β tyrosine kinase [18]. Though sorafenib may provide a modest survival benefit, its broad-spectrum activity also comes with an array of adverse side effects [19]. These side effects that include gastrointestinal bleeding, bone and muscle pain and multiple skin rashes among others may be significant enough to stop the drug, or result in some degree of noncompliance that ultimately dampens its beneficial effects.

Triptolide and Sorafenib Induce Cell Death in HuH-7 and PLC Hepatocellular Carcinoma Cells

Our study showed that triptolide treatment decreased viability in 2 hepatocellular carcinoma cell lines PLC and Huh7 in a time-dependent manner (Figure 2A, B). Cell death was rather notable in the Huh7 cell line, with 85% cell death at 72 hours with a minimal 50 nM concentration of triptolide in combination with 1.25 μ M of sorafenib. The response in the PLC cell line was less robust, although present, with 70% cell death at 72 hours with 50 nM triptolide in combination with 2.5 μ M sorafenib.

Minnelide and sorafenib work in unison to reduce hepatocellular tumors

To see if the Minnelide and Sorafenib combination was as effective *in vivo*, Huh7 cells were implanted in the flanks of athymic nude mice. Once the tumors were approximately 400mm³, they were randomized and divided into four groups: Saline, Sorafenib (10mg/kg body weight), Minnelide (0.42mg/kg body weight) and combination of sorafenib and Minnelide. Animals receiving combination of Minnelide and Sorafenib showed significant tumor regression compared to Saline or either treatment alone (Figure 2C). The tissue sections obtained from the mice receiving treatment when stained with H&E showed similar reduction of tumor tissue (Figure 2C). This experiment confirmed that Minnelide in combination with Sorafenib is indeed a successful combination for treating hepatocellular carcinoma model.

Conclusion

Given the obvious complexity of the interactions between individual tumor cells as well as between the cells and their environment, it would seem a combination drug regimen would be most beneficial in advanced cancer treatments, particularly if agents with different mechanisms of action are combined. The current study showed that Minnelide was a more effective drug against pancreatic cancer models. It effectively reduced tumor burden and tumor related morbidity in different unique but complementary mouse models. It reduced metastatic spread and increased survival in the different models as well. All the above results and observations indicated that Minnelide was a very promising compound in pre-clinical trials that can be taken forward for further development into an effective anti-cancer drug. The Phase I clinical trial of Minnelide in GI tumors is well underway at the University of Minnesota.

Acknowledgments

National Institutes of Health grants R01-CA184274 (to SB) and R01 DK058694 and R01 CA124723, (to A.K.S.) and grants from Robert and Katherine Goodale Foundation and Sit Family foundation (to A.K.S.).

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA: a cancer journal for clinicians. 2010; 60(5):277–300. [PubMed: 20610543]
2. Lowenfels AB, Maisonneuve P. Epidemiology and risk factors for pancreatic cancer. Best practice & research Clinical gastroenterology. 2006; 20(2):197–209. [PubMed: 16549324]
3. O'Sullivan A, Kocher HM. Pancreatic cancer. Clinical evidence. 2007

4. Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet*. 2004; 363(9414):1049–1057. [PubMed: 15051286]
5. McCracken M, Olsen M, Chen MS Jr, Jemal A, Thun M, Cokkinides V, Deapen D, Ward E. Cancer incidence, mortality, and associated risk factors among Asian Americans of Chinese, Filipino, Vietnamese, Korean, and Japanese ethnicities. *CA Cancer J Clin*. 2007; 57(4):190–205. [PubMed: 17626117]
6. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology*. 2013; 144(6):1252–1261. [PubMed: 23622135]
7. Chakraborty S, Baine MJ, Sasson AR, Batra SK. Current status of molecular markers for early detection of sporadic pancreatic cancer. *Biochimica et biophysica acta*. 2011; 1815(1):44–64. [PubMed: 20888394]
8. Binkley, CE.; Simeone, DM. *Pancreatic Cancer*. Elsevier; USA: 2004.
9. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet*. 2012; 379(9822):1245–1255. [PubMed: 22353262]
10. Vincent, A.; Herman, J.; Schulick, R.; Hruban, RH.; Goggins, M. *Lancet*. Vol. 378. England: Elsevier Ltd; 2011. Pancreatic cancer; p. 607-620.
11. Wang, Z.; Li, Y.; Ahmad, A.; Banerjee, S.; Azmi, AS.; Kong, D.; Sarkar, FH. *Nat Rev Gastroenterol Hepatol*. Vol. 8. England: 2011. Pancreatic cancer: understanding and overcoming chemoresistance; p. 27-33.
12. Pancreatic Cancer Detailed Guide. <http://www.cancer.org/cancer/pancreaticcancer/detailedguide/index>
13. Moore, MJ.; Goldstein, D.; Hamm, J.; Figer, A.; Hecht, JR.; Gallinger, S.; Au, HJ.; Murawa, P.; Walde, D.; Wolff, RA., et al. *J Clin Oncol*. Vol. 25. United States: 2007. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group; p. 1960-1966.
14. Saif, MW. New developments in the treatment of pancreatic cancer. *JOP; Highlights from the “44th ASCO Annual Meeting”*; Chicago, IL, USA. May 30 – June 3, 2008; Italy: 2008. p. 391-397.
15. Van Cutsem, E.; Vervenne, WL.; Bennouna, J.; Humblet, Y.; Gill, S.; Van Laethem, JL.; Verslype, C.; Scheithauer, W.; Shang, A.; Cosaert, J., et al. *J Clin Oncol*. Vol. 27. United States: 2009. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer; p. 2231-2237.
16. Llovet JM, Beaugrand M. Hepatocellular carcinoma: present status and future prospects. *Journal of hepatology*. 2003; 38 (Suppl 1):S136–149. [PubMed: 12591191]
17. Gahr S, Wissniowski T, Zopf S, Strobel D, Pustowka A, Ocker M. Combination of the deacetylase inhibitor panobinostat and the multi-kinase inhibitor sorafenib for the treatment of metastatic hepatocellular carcinoma - review of the underlying molecular mechanisms and first case report. *J Cancer*. 2012; 3:158–165. [PubMed: 22514558]
18. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008; 359(4): 378–390. [PubMed: 18650514]
19. Xie B, Wang DH, Spechler SJ. Sorafenib for treatment of hepatocellular carcinoma: a systematic review. *Dig Dis Sci*. 2012; 57(5):1122–1129. [PubMed: 22451120]
20. Strumberg D. Preclinical and clinical development of the oral multikinase inhibitor sorafenib in cancer treatment. *Drugs Today (Barc)*. 2005; 41(12):773–784. [PubMed: 16474853]
21. Lachenmayer A, Toffanin S, Cabellos L, Alsinet C, Hoshida Y, Villanueva A, Minguez B, Tsai HW, Ward SC, Thung S, et al. Combination therapy for hepatocellular carcinoma: additive preclinical efficacy of the HDAC inhibitor panobinostat with sorafenib. *Journal of hepatology*. 2012; 56(6):1343–1350. [PubMed: 22322234]
22. Chugh R, Sangwan V, Patil SP, Dudeja V, Dawra RK, Banerjee S, Schumacher RJ, Blazar BR, Georg GI, Vickers SM, et al. A preclinical evaluation of minnelide as a therapeutic agent against pancreatic cancer. *Science translational medicine*. 2012; 4(156):156ra139.

23. Alsaied OA, Sangwan V, Banerjee S, Krosch TC, Chugh R, Saluja A, Vickers SM, Jensen EH. Sorafenib and triptolide as combination therapy for hepatocellular carcinoma. *Surgery*. 2014; 156(2):270–279. [PubMed: 24953273]
24. Mujumdar N, Mackenzie TN, Dudeja V, Chugh R, Antonoff MB, Borja-Cacho D, Sangwan V, Dawra R, Vickers SM, Saluja AK. Triptolide induces cell death in pancreatic cancer cells by apoptotic and autophagic pathways. *Gastroenterology*. 2010; 139(2):598–608. [PubMed: 20434451]

Figure 1.

(A). Triptolide the active drug reduced cell viability in pancreatic cancer cell line MIA-PACA2. Pancreatic cancer cells were treated with indicated doses of triptolide. Cell viability was assessed by MTT based assay. The X-axis shows the treatment doses while Y-axis shows the cell viability normalized to control. Minnelide has been very effective in pre-clinical models of pancreatic cancer. 200,000 S2-013 cells were injected orthotopically into the tail of the pancreas of athymic nude mice. Treatment with 0.42mg/kg Minnelide (administered daily) was started after 2 weeks of tumor cell injection and continued for 30 days. Tumor weight (B) and tumor volume (C) was measured at the end of study following necropsy. Survival study was performed using pancreatic cancer cell line AsPC-1. 200,000 AsPC1 cells were implanted orthotopically into the tail of the pancreas. Treatment with 0.42mg/kg Minnelide (administered daily) was started after 15 days of tumor cell injection. Median survival of Saline group was 36 days, while all the animals in the Minnelide group survived till the end of study. Further, Minnelide was discontinued after 100 days of treatment (D/C), and the animal were kept under daily observation till the end of study. Tumors did not recur even on discontinuing treatment (D). Resected pancreatic tumor tissue from patients undergoing surgery were implanted subcutaneously in the flank of NOD/SCID mice. Once tumors were ~350mm³, treatment was started with 0.42mg/kg Minnelide(administered daily). Once the tumors in animals of the saline group reached 1000mm³, treatment was started with 0.42mg/kg body weight of animals (administered

daily). Treatment of a patient tumor derived xenograft with Minnelide shows increased survival (E).

(Figure 1D and E are adapted from our original manuscript Chugh et al Sci. Trans. Med. 2012, and the review article Saluja AK, Dudeja V. Relevance of animal models of pancreatic cancer and pancreatitis to human disease. Gastroenterology 2013; 144:1194-8)

* indicated statistically significant data with a p value<0.005; D/C= discontinued; censored data indicate natural death (non-tumor related death) in animals.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2.

Liver cancer cells Huh7 and PLC were treated with indicated doses of triptolide for 24h, 48h and 72h. Cell viability measured by MTT assay, was decreased in combination of triptolide and sorafenib treatment in hepatocellular carcinoma cells Huh7 and PLC (A). For animal experiments, Sorafenib and Minnelide was an effective combination in reducing hepatocellular carcinoma *in vivo* (B) Subcutaneous implants of 1×10^6 HuH7 cell lines were injected subcutaneously in athymic nude mice. When the tumors were 200 mm^3 in size, the animals were randomized into 4 groups: Untreated, Minnelide (0.21mg/kg), Sorafenib (10mg/kg) and a combination of these two drugs. Tumors treated with 0.21mg/kg Minnelide and 10mg/kg Sorafenib showed an increased reduction in tumor volume compared to either drug alone. (B). * indicated statistically significant data with a p value < 0.005