

HIGHLIGHT ARTICLE

Preclinical Research in Treatment of Pancreatic Cancer

Highlights from the "2013 ASCO Annual Meeting". Chicago, IL, USA; May 30 - June 4, 2013

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Summary

Pancreatic adenocarcinoma is an aggressive type of malignancy and remains a treatment-refractory cancer. Because of the few treatment options, understanding of the molecular mechanisms is necessary, for new drugs be developed against molecular targets. Two of the novel, promising regimens against molecular targets, NVP-BE235 and MSK-777, were examined in three preclinical studies performed in human pancreatic cell lines and mouse models and presented in the 2013 ASCO Annual Meeting. Two of the studies evaluated the role of NVP-BE235, an oral phosphatidylinositol-3-kinase (PI3K) inhibitor, in pancreatic cancer treatment, alone and in combination with nab-paclitaxel (Abstract #e15007) or gemcitabine (Abstract #e15070). The third study presents the effectiveness of the novel cell division cycle 7 (Cdc7) kinase inhibitor, MSK-777 (Abstract #e15059). All studies demonstrated promising results and further investigation is ongoing.

What Did We Know Before the 2013 American Society of Clinical Oncology (ASCO) Annual Meeting?

Exocrine pancreatic cancer is one of the most aggressive types of malignancy [1]. At the time of diagnosis, most of patients present with advanced or metastatic disease [2]. Gemcitabine has been considered as the standard treatment for advanced pancreatic cancer since 1997 [3]. However, its efficacy remains moderate and the median overall survival times range from 5 to 8 months. Numerous studies have attempted to increase efficacy of chemotherapy by combining gemcitabine with other agents, but only the combination with erlotinib seems to induce a modest but significant increase in overall survival [4]. In metastatic disease FOLFIRINOX (5-fluorouracil, oxaliplatin, irinotecan, leucovorin) has shown superiority over gemcitabine [5] while, recently in 2013, there were

encouraging results in MPACT study that showed an improvement in overall survival using nab-paclitaxel plus gemcitabine *versus* gemcitabine alone [6].

Nevertheless, pancreatic adenocarcinoma remains a treatment-refractory cancer and its management remains one of the most challenging in oncology. During the last few years there have been important advances to better understand the molecular base in the development of pancreatic adenocarcinoma [7]. The sequential acquisition of mutations in the proto-oncogene *K-ras* and the tumor suppressors *INK4A*, *TP53* and *DPC4/SMAD4* and their accumulation leads to a profound disturbance in cell cycle regulation and continuous growth [1].

Abnormal signalling by phosphatidylinositol-3-kinase (PI3K) is a prominent feature of pancreatic cancers, due to the high prevalence of abnormalities that regulate this pathway, including *K-ras* mutations that occur in approximately 90% of cases [8]. It is likely that the activation of this pathway likely has an important role on the aggressive clinical features of pancreatic cancer, including resistance to chemotherapy [9]. Genetic alterations, activating *K-ras* and PI3K/AKT signalling, are also known to induce the activity of mammalian target of rapamycin (mTOR) kinase in pancreatic adenocarcinoma (Figure 1) [10]. Targeting the PI3K-AKT-mTOR pathway could arrest tumor growth and

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Abbreviations Cdc7: cell division cycle 7; MCM: mini-chromosome maintenance complex; NVP-BE235: dactolisib; PI3K: phosphatidylinositol-3-kinase

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induce cell death in cancers that are resistant to currently available therapies. NVP-BEZ235 (dactolisib; Pharma, Basel, Switzerland) is a synthetic low molecular mass compound belonging to the class of imidazoquinolines [11]. It is an oral targeted anticancer agent that shows high selectivity for class I PI3K and the structurally related mTOR [12].

Also, studies on the molecular basis of various diseases have revealed that the deregulation of protein phosphorylation reactions by kinases is associated with many diseases, especially cancer [13]. Cell division cycle 7 (Cdc7)-related protein kinase is a heterodimer of a kinase and its activator (Dbf4). It is essential for the activation of the minichromosome maintenance complex (MCM2-7), the helicase that unwinds the strands of DNA during replication (Figure 2) [14, 15]. Cdc7 is a target of the S-phase checkpoint, and has a role in controlling the DNA damage response. Inhibition of Cdc7 in cancer cells seems to result in slowing of S-phase and cell cycle arrest, followed by accumulation of DNA damage [13]. Cdc7 has been shown to be over-expressed in many different tumors including the majority of solid and liquid tumors. A novel natural product small molecule inhibitor (MSK-777) has been identified, developed and shown to be highly potent in both cell based and animal models of cancer [14].

What Have We Learnt from the American Society of Clinical Oncology (ASCO) Annual Meeting, 2013?

In the 2013 ASCO Annual Meeting were presented two abstracts concerning preclinical

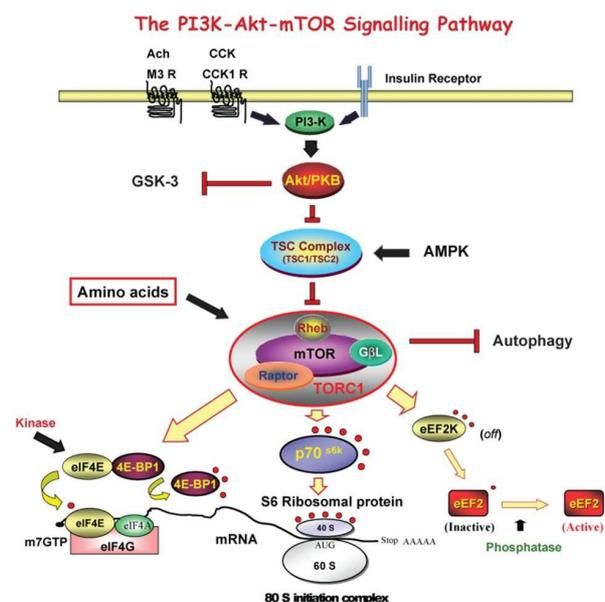


Figure 1. PI3K-AKT-mTOR signalling pathway. (From: Sans Gili MD, <http://www.pancreapedia.org> 2010. The copying and use of the content of Pancreapedia do not infringe the proprietary rights according to the "Terms of Service").

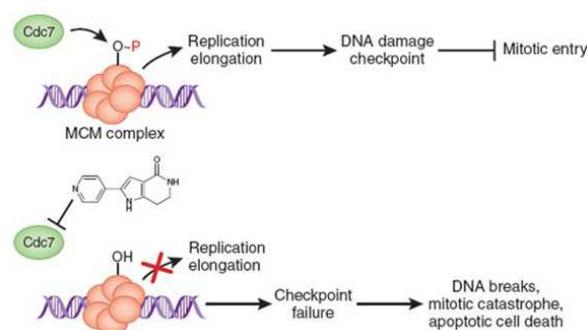


Figure 2: Cdc7 regulation of DNA replication and checkpoints in normal and tumor cells. With permission from Jackson 2008 [15].

studies for the effectiveness of the agent NVP-BEZ235 (Abstracts #e15007, #e15070) and one study for the role of MSK-777 in pancreatic cancer (Abstract #e15059).

Preclinical Study of Cytotoxicity and Predictive Markers of Response to Dual Inhibition of PI3K and mTORC1/2 Signaling by NVP-BEZ235 With or Without Paclitaxel or Nab-Paclitaxel as a New Therapeutic Strategy in Pancreatic Cancer Cell Lines (Abstract #e15007) [16]

Loaiza-Bonilla *et al.* performed a preclinical study using K-ras mutant and wild-type human pancreatic cell lines *in vitro*. They treated these cell lines with NVP-BEZ235 and with its combination with nab-paclitaxel. The Western blot analysis was used for S235/S236P-RPS6 and Akt (S473P-Akt, T308P-Akt) levels after the treatment. They found that although NVP-BEZ235 reduced S473P-Akt, T308P-Akt and S235/S236P-RPS6, its combination with nab-paclitaxel had a significant synergistic effect in a time- and dose-dependent fashion in comparison with the corresponding single drug treatments. These results concern only the wild-type K-ras human pancreatic cell lines (BxPC-3) but not the K-ras mutant cell lines (MIA PaCa-2 and PANC-1), where there was a paradoxical increase in proliferation.

Effect of the Combination of the Dual mTOR/PI3K Inhibitor NVP-BEZ235 with Gemcitabine on Growth Inhibition in Pancreatic Cancer Cells In Vitro and In Vivo (Abstract #e15070) [17]

Maute *et al.* examined the efficacy of NVP-BEZ235 alone or in combination with gemcitabine both *in vitro* in human pancreatic cell lines (MiaPaCa-2, Panc-1, AsPC-1 and BxPC-3) and *in vivo*, in mouse models of pancreatic exocrine neoplasia (NOD SCID mice). This study showed that, *in vitro*, the simultaneous administration of NVP-BEZ235 and gemcitabine was more effective than the single agent administration but the best results were obtained with the sequential administration of gemcitabine followed by NVP-BEZ235. *In vivo*, the

most effective administration was the simultaneous and even better was the sequential administration of gemcitabine followed by NVP-BEZ235. Single agents (gemcitabine and NVP-BEZ235) and in the sequence NVP-BEZ235 followed by gemcitabine, resulted in a minimal reduction of tumor growth.

Cdc7 Inhibition as a Novel Approach for Pancreas Cancer Therapy (Abstract #e15059) [18]

Frattini *et al.* examined the effectiveness of MSK-777 in pancreatic cancer. They used three cell lines, Capan-1, BxPC3, and PANC-1 and cytotoxicity of the regimen was estimated. MSK-777, control (dimethyl sulfoxide; DMSO), or hydroxyurea was administered. After the first 24 hours in BxPC3 cells there was a dramatic effect with reduced cell viability to less than 20%. Also, the study demonstrated that in Capan-1 and PANC-1 cells, the administration of MSK-777 resulted in cell cycle arrest at G1/S by 48 hours while in the BxPC3 cells, there was a significant sub-G1 population by 24 hours, indicating apoptotic cell death. Concerning the phosphorylation of MCM-2, in BxPC3 cells it disappeared by 24 hours, indicating inactivation of the helicase that unwinds the strands of DNA during replication, while in Capan-1 and PANC-1 cells there were lower levels of phosphorylated MCM-2 by 48 hours

Discussion

Pancreatic cancer represents the fifth leading cause of cancer death in the Western population with a five-year survival rate under 5% [19]. Because of the few treatment options, understanding of the molecular mechanisms is prerequisite to identify potential molecular targets for drug therapy [1]. The identification of novel molecular targets is critical in the development of new and efficient cancer therapies [13]. Two promising regimen, NVP-BEZ235 and MSK-777 were presented in ASCO 2013 Annual Meeting

Concerning the role of NVP-BEZ235 in treatment of pancreatic adenocarcinoma, only a few preclinical studies have been published. The first of them, performed in 2009, showed that this agent might have anticancer activity but the authors supported the testing of combination of NVP-BEZ235 with other anticancer agents [9]. Also, more recent studies using NVP-BEZ235, demonstrated that it is unlikely for the complete target inhibition to be sustained over long periods, as PI3K signalling is critical in the maintenance of normal tissues. So combination of this agent with other anticancer agents was studied [10, 20]. So, in 2012, two preclinical studies showed that NVP-BEZ235 alone could effectively induced apoptosis and reduced tumor growth, but the effectiveness was further enhanced by combination of gemcitabine and

endothelial monocyte activating polypeptide II (EMAP) [20] or by combination with the pan-histone deacetylase inhibitor panobinostat (PS) [10]. In agreement with these results, Loaiza-Bonilla *et al.* showed that NVP-BEZ235 in combination with nab-paclitaxel had a significant synergistic effect compared to monotherapy [16]. This effectiveness was demonstrated only in cancer cell lines with mutant *K-ras* and not in wild type. The authors resumed that *K-ras* may be studied as a potential predictive biomarker. The second study concerning the effectiveness of NVP-BEZ235 was performed from Maute *et al.* [17] and they used the combination of NVP-BEZ235 of gemcitabine. They used not only *in vitro* analysis (cell lines) but also *in vivo* experiments in NOD SCID mice. Although, the combination of the two regimens seemed to work significantly better than the single agents, it is interesting that the most effective was the sequential administration of gemcitabine followed by NVP-BEZ235, in both *in vitro* and *in vivo* analysis. The above promising results may offer the opportunity of a more effective treatment of pancreatic cancer.

Cdc7 is overexpressed in most cancers, including many solid tumors and acute leukemias. For the *Cdc7* inhibitor MSK-777, examined in the study by Frattini *et al.* [18], there are not published data until today, as it is a novel regimen identified in the researchers' laboratory, while their first efforts focused in treatment of leukemia [14]. Studies have shown that *Cdc7* inhibition in cancer cells leads to p53-independent apoptotic cell death, as it produces cells bearing incompletely and/or abnormally replicated DNA, which results in cell death [21]. It is interesting that *Cdc7* depletion does not elicit apoptotic cell death in normal human diploid cells, as it seems that there is a cell-cycle check-point mechanism [21]. The preclinical data showed that *Cdc7* is a novel and promising target for tumor cell killing but ongoing trials will reveal whether inhibition of this kinase represents a successful strategy that could bring benefits to cancer patients in terms of superior activity and better tolerability compared with the currently approved agents [13, 21].

Conclusions

In conclusion, although during the last few years there have been important advances in the understanding of the molecular events responsible for the development of pancreatic cancer, currently specific mechanisms of treatment resistance remain poorly understood and new effective systemic drugs need to be developed and probed. The results of the above presented studies in 2013 ASCO Annual Meeting, show that NVP-BEZ235 and MSK-777 represent novel and promising treatment, although they have to be tested in further studies.

Conflicts of interest No conflict to disclose

References

1. Schneider G, Schmid RM. Genetic alterations in pancreatic carcinoma. *Mol Cancer*. 2003; 2: 15.
 2. Altwegg R, Ychou M, Guillaumon V, Thezenas S, Senesse P, Flori N, et al. Second-line therapy for gemcitabine-pretreated advanced or metastatic pancreatic cancer. *World J Gastroenterol*. 2012; 18: 1357-64.
 3. Burris HA, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; 15: 2403-2413.
 4. Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, et al. 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. *J Clin Oncol* 2007; 25: 1960-1966.
 5. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; 364: 1817-1825.
 6. Von Hoff DD, Ervin TJ, Arena FP, Chiorean EG, Infante JR, Moore MJ, et al. Randomized phase III study of weekly nab-paclitaxel plus gemcitabine versus gemcitabine alone in patients with metastatic adenocarcinoma of the pancreas (MPACT). *J Clin Oncol* 30: 2012 (suppl 34; abstr LBA148).
 7. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; 321: 1801-1806.
 8. Agbunag C, Bar-Sagi D. Oncogenic K-ras drives cell cycle progression and phenotypic conversion of primary pancreatic duct epithelial cells. *Cancer Res* 2004; 64: 5659-5663.
 9. Cao P, Maira SM, García-Echeverría C, Hedley DW. Activity of a novel, dual PI3-kinase/mTOR inhibitor NVP-BEZ235 against primary human pancreatic cancers grown as orthotopic xenografts. *Br J Cancer*. 2009; 100: 1267-76.
 10. Venkannagari S, Fiskus W, Peth K, Atadja P, Hidalgo M, Maitra A, Bhalla KN. Superior efficacy of co-treatment with dual PI3K/mTOR inhibitor NVP-BEZ235 and pan-histone deacetylase inhibitor against human pancreatic cancer. *Oncotarget*. 2012; 3: 1416-27.
 11. Serra V, Markman B, Scaltriti M, Eichhorn PJ, Valero V, Guzman M, et al. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res*. 2008; 68: 8022-30.
 12. Maira SM, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, et al. Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. *Mol Cancer Ther* 2008; 7: 1851-1863
 13. Sawa M, Masai H. Drug design with Cdc7 kinase: a potential novel cancer therapy target. *Drug Des Devel Ther*. 2009; 2: 255-64.
 14. Davis J, Zhang R, Frattini MG. Synergy between conventional chemotherapy and Cdc7 inhibition as a novel approach for cancer therapy. *Molecular Cancer Therapeutics* 2011; 10 (suppl 1; abstr75).
 15. Jackson PK. Stopping replication, at the beginning. *Nat Chem Biol*. 2008; 4: 331-2.
 16. Loaiza-Bonilla A, Kittaneh M, Guardiola Amado VD, Kovacs K, Merchan JR. Preclinical study of cytotoxicity and predictive markers of response to dual inhibition of PI3K and mTORC1/2 signaling by NVP-BEZ235 with or without paclitaxel or nab-paclitaxel as a new therapeutic strategy in pancreatic cancer cell lines. *J Clin Oncol* 31, 2013 (suppl; abstr #e15007).
 17. Maute L, Wicht J, Zoernig M, Niederhagen M, Bergmann L. Effect of the combination of the dual mTOR/PI3K inhibitor NVP-BEZ235 with gemcitabine on growth inhibition in pancreatic cancer cells in vitro and in vivo. *J Clin Oncol* 31, 2013 (suppl; abstr #e15070).
 18. Frattini MG, Regales L, Santos R, Carrillo D. Cdc7 inhibition as a novel approach for pancreas cancer therapy. *J Clin Oncol* 31, 2013 (suppl; abstr #e15059).
 19. Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, 2002. *CA Cancer J Clin* 2002; 52: 23-47.
 20. Awasthi N, Yen PL, Schwarz MA, Schwarz RE. The efficacy of a novel, dual PI3K/mTOR inhibitor NVP-BEZ235 to enhance chemotherapy and antiangiogenic response in pancreatic cancer. *J Cell Biochem*. 2012; 113: 784-91.
 21. Montagnoli A, Moll J, Colotta F. Targeting cell division cycle 7 kinase: a new approach for cancer therapy. *Clin Cancer Res*. 2010; 16: 4503-8.
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