RESEARCH ARTICLE

Editorial Process: Submission:05/13/2023 Acceptance:08/07/2023

The Mutation Portraits of Oncogenes and Tumor Supressor Genes in Predicting the Overall Survival in Pancreatic Cancer: A Bayesian Network Meta-Analysis

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Abstract

Introduction: In pancreatic cancer, the carcinogenesis can not be separated from genetics mutations. The portraits of genes alterations majorily including oncogenes (KRAS, HER2, PD-L1) and tumor supressor genes (P53, CDKN2A, SMAD4). Besides being notorious a screening marker, the genetic mutations were related to the prognosis of pancreatic cancer. The aim of this study is to determine the genetic mutations portrait in predicting the overall survival in pancreatic cancer. **Methods:** The network meta analysis (NMA) was registered in PROSPERO (CRD42023397976) and conducted in accordance with the PRISMA-P (Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols) in addition of NMA extension guidance. Comprehensive searches were done including all studies which reported the overall survival of pancreatic cancer subjects with KRAS, HER2, PD-L1, P53, CDKN2A, SMAD4. Data were collected and analysis will be done based on Bayesian method, Markov Chain Monte Carlo algorithm, using BUGSnet package in R studio. Transivity was controlled by methods and consistency of the NMA will be fitted by deviance information criterion. Data analysis in NMA were presented in Sucra plot, league table, and forest plot. **Results:** Twenty-four studies were included in this NMA with 4613 total subjects. The NMA was conducted in random-effects, consistent, and convergence model. Relative to control, the genetic mutation of SMAD4 (HR 1.84; 95%CI 1.39-2.46), HER2 (HR 1.76; 95%CI 1.14-2.71), and KRAS (HR 1.7; 95%CI 1.19-2.48) were significant to have worse survival. The mutations of PD-L1, P53, and CDKN2A also showed poor survival, but not statistically significant compared to control. **Conclusion:** In pancreatic cancer, the mutation of SMAD4 predicted the worst overall survival, compared to control, also mutation of HER2, KRAS, PD-L1, P53, and CDKN2A.

Keywords: Pancreatic cancer- overall survival- genetic mutation

Asian Pac J Cancer Prev, **24 (8)**, 2895-2902

Introduction

Pancreatic cancer is one of the most fatal cancer of cases of malignancies (Carrato et al., 2015). The latest cancer worldwide data from Globocan (2020) reported that there were 495,773 (2.6%) new cases and 466,003 (4.7%) death cases of pancreatic cancer worldwide (Sung et al., 2021). The reported 5-year survival rate of pancreatic cancer accounted for only less than 10% in the USA and still increasing (Mizrahi et al., 2020). Despite advancement in the knowledge of pancreatic cancer diagnosis dan treatment, its incidence is increasing to 355,317 new cases in 2040 (Rawla et al., 2019).

About 70-90% subjects with pancreatic cancer have gene alterations (Cicenas et al., 2017). The most commonly mutated genes were KRAS, P53, SMAD4, and CDKN2A, as summarised from The Cancer Genome Atlas (Bailey et al., 2016). Further, study with whole-genome sequencing has revealed that the main driver genes in pancreatic cancers includes HER2 and PD-L1 (Waddell et al., 2015). Those mutated genes are found to affect different stages pancreatic cancer carcinogenesis, promoting the differentiation and proliferation of pancreatic cancers cells (Hu et al., 2021). KRAS mutation was founded in 70-95% (Bamford et al., 2004), p53 mutation in 20-76% (Rice and Del Rio Hernandez, 2019), CDKN2A mutation 49-98% (Chen et al., 2009), SMAD4 mutation in 19-50% (De Bosscher et al., 2004), HER2 overexpression in 0-82% (Han et al., 2021), and PD-L1 mutation in 41% subjects (Zhao and Cao, 2020). Most studies used genetic

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mutation data as a marker in screening or diagnosis for pancreatic cancer. Study analyzing the relationship of genetic mutation to predict pancreatic cancer survival was still limited. In addition, most studies only compared one genetic mutation per study, thus the generalization of which genetic mutation confered the worst prognosis in pancreatic cancer could not be determined. The aim of this study was to determine which genetic mutation in pancreatic cancer predicted the worst overall survival. Thus, more aggressive measures could be considered in advance.

Materials and Methods

Study design

The network meta analysis (NMA) was conducted in accordance with the PRISMA-P (Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols) in addition of NMA extension guidance (Moher et al., 2015). The literature search was established to address the research question phrased as follows in the PICO framework: Population (subjects with pancreatic cancer), Interventions (Mutation of KRAS, P53, SMAD4, CDKN2A, HER2, PD-L1), Comparison (Control), Outcome (Overall survival (OS)). This study has been registered in PROSPERO with registration number CRD42023397976.

Literature search and study selection

We searched Cochrane CENTRAL, PubMed/Medline, Embase, and Web of Science using a search strategy using all available keywords related to this study. The search consisted of four domains (intervention, outcome, methodology, and some exclusion terms) and medical subject headings were used for searching PubMed/Medline. We also searched for the citation in each study to obtain more studies.

The articles were updated to 12th January 2023. All randomized clinical trials (RCTs), quasi-RCTs, and observational studies were eligible, but no RCTs were identified. We did not restrict on date, publication status, or year of publication. The inclusion criteria were (1) studies comparing overall survival between subjects, (2) fulfilled the good study criteria according to to the GRADE Working Group, (3) Use resection specimen or EUS-FNA for immunohistochemitry or next-generation sequencing examination. The exclusion criteria were (1) study conducted in mice, (2) no exact survival data or Kaplan Meier plot. Then, a standardized electronic data form in Microsoft Excel will be used to extract the following data: author name, country, year of study, stage, specimen, examination, gender, age, median survival, genetic mutation, and overall survival. Extracted data from included studies by two independent reviewers to reduce bias and a third one verified the data to avoid repeat inclusion.

Risk of bias assessment

Studies will be assessed for bias using the Cochrane risk of bias tool considering the judgment of the random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias as "Low risk" of bias, "High risk" of bias, or "Unclear risk" of bias (Barcot et al., 2019).

Network meta analysis

The network meta-analysis was conducted using a Bayesian method using the BUGSnet package of R software (Béliveau et al., 2019). Network meta-analysis was an indirect method in comparing various variables that were not compared head to head in observational studies. The NMA model was done on a Bayesian approach through the Markov Chain Monte Carlo (MCMC) simulation. We specified the Bayesian framework with 1,000 number of adaptations, 1,000 burn-ins, and 10,000 iterations. Model selection and goodness-of-fit were evaluated through deviance information criteria (DIC). Adequacy of the model fit was assessed through a comparison of the residual deviance of the models, where a close match between both models was considered an adequate fit.

To rank the treatment, we conducted a SUCRA plot, expressed as a percentage, is the relative probability of an intervention being among the best options or better than other interventions. League table was shown to determined the relative hazard ratio among comparisons. To ensure that convergence was reached, the Brooks-Gelman-Rubin statistic was assessed.

Results

The literature search process is shown in Figure 1. Following removal of duplicates, 2,713 records were screened on the basis of titles and abstracts. Full-text assessment was performed for 30 articles. Based on the determined inclusion criteria, 29 articles were included in the eligibility full-text analysis. The reason for exclusion of the other 5 articles is presented. Finally, 24 articles were included for further analysis. The characteristics and information of the included studies are shown in Table 1. There were 7 interventions in 24 studies with 4613 total number of patients in the network. The mean follow up time in this NMA was 2.58 years. Figure 2 showed the network of treatment comparisons in available trials.

The analysis model used in this study is a random model. The DIC is considerably lower in the random effects model. The fixed effects model shows that 5 points are largely contributing to the model's poor fit. The random effects model appears to have only 1 outlier, which should be investigated (Figure 3a and 3b). Next, we will assess consistency in the network by fitting a random effects inconsistency model and comparing it to our random effects consistency model. With the exception of 1 or 2 points, the data lies on y and x line, indicating a general agreement between the two models (Figure 3c). This suggests that we proceed with the consistency and random effects model.

In a Bayesian framework, ranks may be determined based on the mean or median of the posterior distribution of the ranks, presented in a numeric presentation of the overall ranking in the Surface Under the Cumulative Ranking curve (SUCRA). The higher the SUCRA value,

Table 1. Characteristics of Studies Included in This Network Meta-Analysis

Author	Country	Male/ Female	Stage	Specimen	Exam-ination	Age	Genetic mutation	Median survival
Bachet (2012) (Bachet et al., 2012)	France	245/208	IA-IIB	Resection tissue	IHC	34-88	SMAD4	30
Birnbaum (2016) (Birnbaum et al., 2016)	France	154/128	I-IV	Resection tissue	IHC	32-84	PD-L1	6.5
Blackford (2009) (Blackford et al., 2009)	USA	43/46	I-IV	Resection tissue	NGS	36-85	P53 SMAD4	NA
Han (2021) (Han et al., 2021)	South Korea	27/28	I-IV	Resection tissue	IHC	31-81	HER2	NA
Hua (2003) (Hua et al., 2003)	China	22/12	I-IV	Resection tissue	IHC	30-75	SMAD4	-
Iwatate (2020) (Iwatate et al., 2020)	Japan	59/44	I-III	Resection tissue	NGS	50-87	CDKN2A	22
Jiang (2012) (Jiang et al., 2012)	China	60/102	I-IV	Resection tissue	IHC	34-85	CDKN2A SMAD4	NA
Kinugasa (2015) (Kinugasa et al., 2015)	Japan	54/21	I-IV	EUS-FNA	NGS	47-85	KRAS	24
Komoto (2009) (Komoto et al., 2009)	Japan	NA	I-IV	Resection tissue	IHC	NA	HER2	NA
Liang (2018) (Liang et al., 2018)	China	215/158	I-IV	Resection tissue	IHC	29-82	PD-L1	20
McIntyre (2021) (McIntyre et al., 2020)	USA	137/146	I-IV	Resection tissue	IHC	59-73	P53	39
Oshima (2013) (Oshima et al., 2013)	Japan	62/44	I-IIB	Resection tissue	IHC	36-86	CDKN2A P53	22
Ottenhof (2012) (Ottenhof et al., 2012)	Nether-lands	35/43	I-III	Resection tissue	IHC	40-77	SMAD4	27
Principe (2022) (Principe et al., 2022)	USA	NA	I-IV	Resection tissue	WB	NA	SMAD4	23
Saxby (2005) (Saxby et al., 2005)	Australia	17/13	I-III	Resection tissue	IHC	39-83	HER2	NA
Schultheis (2017) (Schultheis et al., 2017)	Germany	NA	NA	Resection tissue	NGS	NA	KRAS	8
Sharif (2008) (Sharif et al., 2008)	USA	27/33	I-IV	Resection tissue	IHC	28-85	HER2	18.5
Shen (2022) (Shen et al., 2022)	Australia	109/122	I-IV	Resection tissue, EUS-FNA	NGS	NA	KRAS	20
Shin (2013) (Shin et al., 2013)	South Korea	161/111	I-IV	Resection tissue	NGS	22-78	CDKN2A	16
					IHC		HER2 KRAS P53	
Shin (2017) (Shin et al., 2017)	South Korea	374/267	I-IV	Resection tissue	IHC	22-84	SMAD4	20
Wang (2010) (Wang et al., 2010)	China	50/31	I-III	Resection tissue	IHC	34-76	PD-L1	24
Wang (2017) (Wang et al., 2017)	China	63/51	I-IV	Resection tissue	IHC	31-78	PD-L1	14
Windon (2018) (Windon et al., 2018)	USA	25/14	I-IV	Resection tissue, EUS-FNA, Core needle biopsy	NGS	NA	KRAS	17
Yamaki (2017) (Yamaki et al., 2017)	Japan	26/16	I-III	Resection tissue	IHC	50-83	PD-L1	26

and the closer to 100%, the higher the likelihood that a variable had better outcome (Salanti et al., 2011). In this study, from the SUCRA plot, it was shown that the genetic mutation that had the worst OS was loss of SMAD4, followed by HER2 overexpression, KRAS mutant, positive PD-L1, P53 mutation, and the best was when a CDKN2A mutation was found (Figure 4).

League tables contain all information about relative effectiveness for all possible pairs of interventions with 95% confidence interval. The values in the below league table report a hazard ratio scale (Austin et al., 2017). In this study specifically, it was shown that subjects without gene mutations had the highest overall survival. Subjects with SMAD4 mutations showed the worst OS (HR 1.84; 95%CI 1.39-2.46) whereas subjects with CDKN2A mutations (HR 1.09; 95%CI 0.77-1.57) showed better OS ramong others genetic mutations (Figure 5). Of all the genetic mutations, only KRAS, HER2, and SMAD4 showed a significantly worse prognosis than controls. Forest plot was also shown below to summarize the results of an NMA with respect to a particular comparator (Figure 6). This model was proved to be convergence in Brooks-Gelman-Rubin statistic testing (Figure 7).

Discussion

Genetic mutations have become a pivotal variables in recent studies about pancreatic cancer (Idachaba et al.,

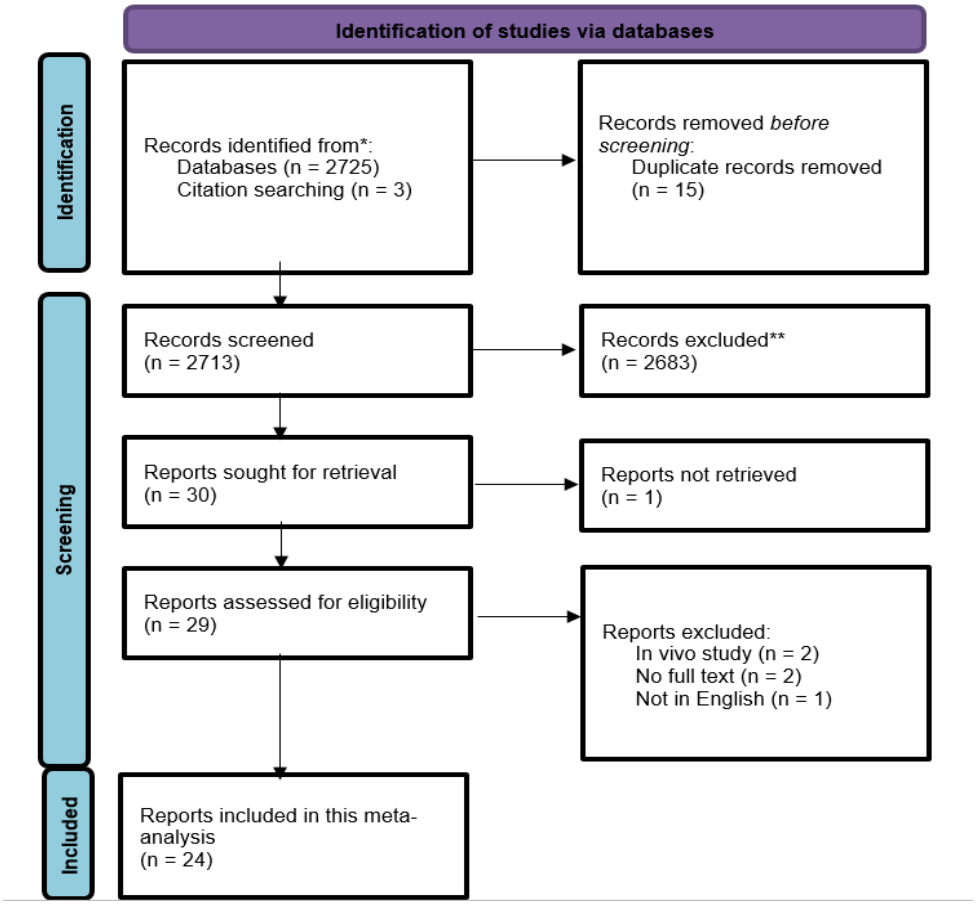

Figure 1. PRISMA Flow Chart. The systematic review and network meta-analysis was conducted according to the guidelines recommended by PRISMA

2019). Unbalanced oncogenes and tumor supressor genes promotes the development of pancreatic cancer disease, thus cell cycle progressing without any inhibitory function (Abramson et al., 2007). The carcinogenesis of pancreatic cancer starts from the development of precancerous cells, pancreatic intraepithelial neoplasias (PanINs) and intraductal papillary mucinous neoplasms. Ductal epithelial cells with KRAS mutation will rapidly progess to PanIN-1, then to PanIN-2 due to CDKN2A mutation. Further accumultation of P53 and SMAD4 mutations will progress the cancer cells to PanIN-3, then pancreatic ductal adenocarcinomas (Grant et al., 2016). Many genetic mutations also contributed for the carcinogenesis of pancreatic cancer, such as PD-L1 and HER2.

In this study, it was shown that loss of the SMAD4 tumor suppressor gene showed the worst overall survival

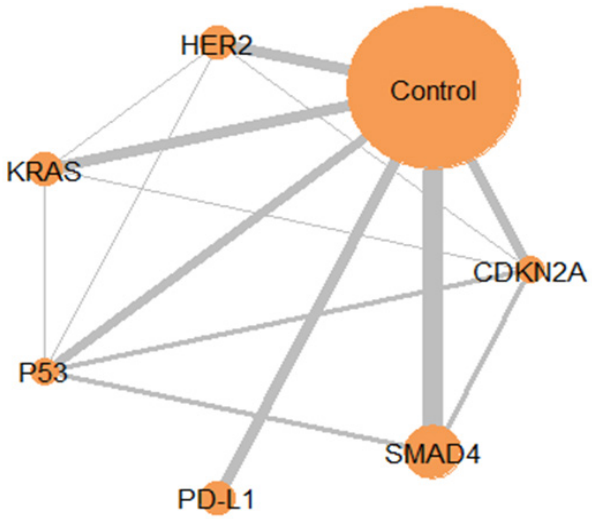

Figure 2. Network Plot of Trials Evaluating the Genetic Mutations Affecting Pancreatic Cancer Survival. The size of each circles were proportional to the number of subjects in each genetic mutations. The width of lines were proportional to the number of trials comparing the corresponding pair of genetic mutations.

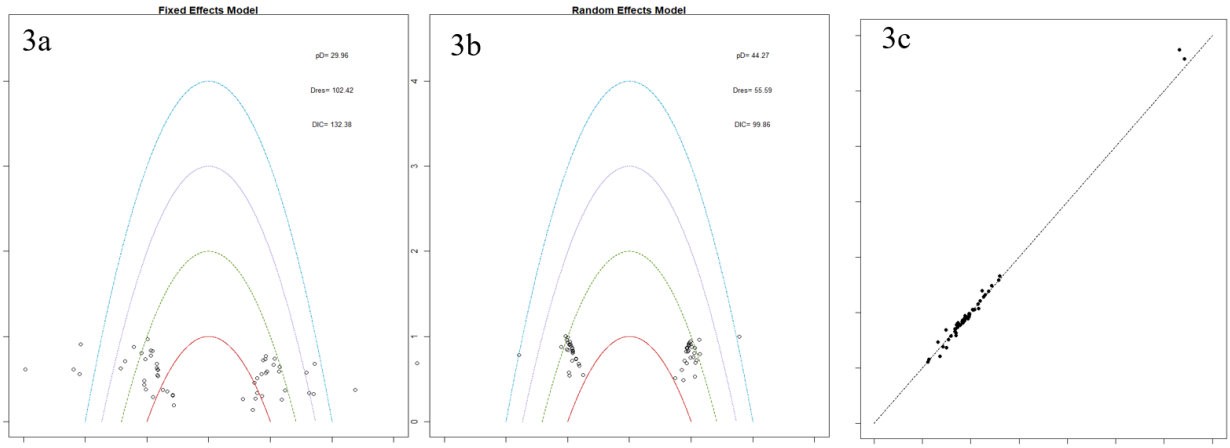

Figure 3. a, fixed-effects model; b, random-effects model; c, consistency and inconsistency agreement

compared to other tumor suppressor mutations such as CDKN2A and p53 which showed better overall survival. It is known that SMAD4 is an important player in the development of PanIns into PDAC. The SMAD4 gene encodes a cytoplasmic mediator of the transforming growth factor-beta (TGF-beta) signaling pathway. serves an important tumor suppressor function. Loss of SMAD4 is associated with carcinogenesis and the poor survival (Principe et al., 2022). Although p53 plays a cardinal role as a tumor suppressor, causing cells to proliferate without control, it has been found that the prognosis is much better than SMAD4 mutations (Voutsadakis, 2021). CDKN2A is a tumor suppressor gene that encodes the p16INK4A protein (hereafter mentioned as CDKN2A). As it names, CDKN2A is a negative regulator of cell cycle progression (G1-to-S phase transition) by disturbing the complex formation between CDK4/6 and cyclin D. CDKN2A is frequently inactivated in cancers due to genetic alterations by point mutation, homozygous deletion, promoter hypermethylation, and loss of heterozygosity, which also contributed in pancreatic cancer (Lin et al., 2020).

RAS genes (HRAS, KRAS, and NRAS) show the most frequent propensity of genetic mutations that promote pancreatic cancer progession. In normal condition, RAS is predominantly bound to GDP as an inative from. Upon stimulated by receptor tyrosine kinases (RTKs) and other cell-surface receptors, RAS-GTP formed, leading to engagement of effector proteins that then regulate a diversity of intracellular signaling networks and thereby tightly control mitogenic processes (Waters and Der, 2018). HER2, also known as ERBB2, is a receptor tyrosine kinase that promotes cell growth and proliferation. This gene expression is associated with poor clinical outcomes in pancreatic cancer (Shibata et al., 2018). Programmed death-ligand 1 (PD-L1) is an immune checkpoint inhibitor that binds to PD-1 receptor to promote cells growth, which also associated with poor outcomes (Karamitopoulou et al., 2021). In this NMA, oncogenes such as PD-L1, KRAS, and HER2 have a better prognosis than loss of SMAD4, but still worse than loss of P53 or CDKN2A. Of the three

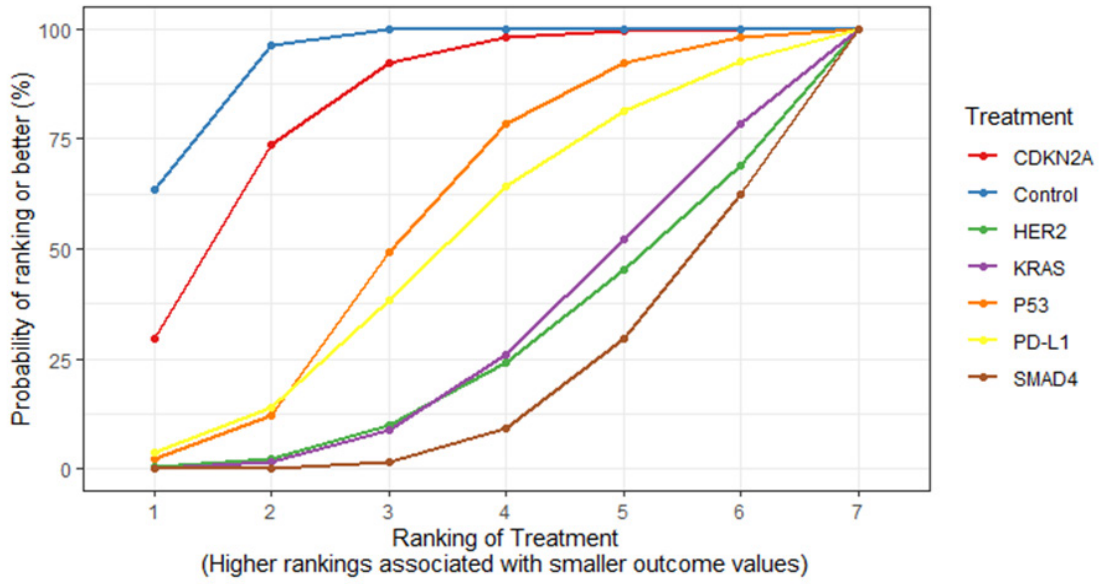

Figure 4. Surface under the Cumulative Ranking Curve (SUCRA) Plot to Rank the Best to Worst Overall Survival in Patients with each Genetic Mutation. The more the curve for a certain strategy is located toward the upper left corner, the higher its SUCRA value, and the better prognosis it has.

Comparator \ Treatment	Control	CDKN2A	P53	PD-L1	KRAS	HER2	SMAD4
Control		1.09 (0.77, 1.57)	1.37 (0.96, 1.97)	1.44 (0.93, 2.20)	**1.70** (1.19, 2.48)	**1.76** (1.14, 2.71)	**1.84** (1.39, 2.46)
CDKN2A	0.92 (0.64, 1.31)		1.26 (0.81, 1.95)	1.32 (0.75, 2.30)	1.56 (0.97, 2.53)	1.61 (0.94, 2.73)	**1.69** (1.12, 2.51)
P53	0.73 (0.51, 1.04)	0.80 (0.51, 1.23)		1.05 (0.59, 1.82)	1.24 (0.77, 2.02)	1.28 (0.75, 2.19)	1.34 (0.89, 2.03)
PD-L1	0.69 (0.46, 1.07)	0.76 (0.43, 1.33)	0.95 (0.55, 1.69)		1.18 (0.67, 2.11)	1.22 (0.67, 2.24)	1.28 (0.77, 2.16)
KRAS	**0.59** (0.40, 0.84)	0.64 (0.40, 1.03)	0.81 (0.49, 1.30)	0.85 (0.47, 1.49)		1.03 (0.60, 1.77)	1.08 (0.68, 1.70)
HER2	**0.57** (0.37, 0.88)	0.62 (0.37, 1.06)	0.78 (0.46, 1.33)	0.82 (0.45, 1.49)	0.97 (0.56, 1.67)		1.05 (0.63, 1.76)
SMAD4	**0.54** (0.41, 0.72)	**0.59** (0.40, 0.89)	0.74 (0.49, 1.12)	0.78 (0.46, 1.29)	0.92 (0.59, 1.46)	0.95 (0.57, 1.59)	

Figure 5. League Tables Showing the Results of the Network Meta-Analyses Comparing the Overall Survival of all Genetic Mutations Including Hazard Ratios (HR) and 95% Confidence Intervals. In this table, green cell indicates that a gene mutation had better prognosis than its comparator (estimate smaller than 1), while a red cell indicates that the gene mutation had worst prognosis than its comparator (estimate greater than 1). The symbols (**) are used to highlight credible intervals that do not contain the neutral value 1, meaning that there is evidence of a statistically significant difference between the variables and its comparator at the 95% confidence level.

oncogenes, HER2 does not show a better prognosis, although it is almost equal to KRAS. So, even though the incidence of KRAS gene mutations is found to be higher, the prognosis is slightly better than HER2.

Our study has several limitations. First is excluding the non-English language articles. Second, not all study

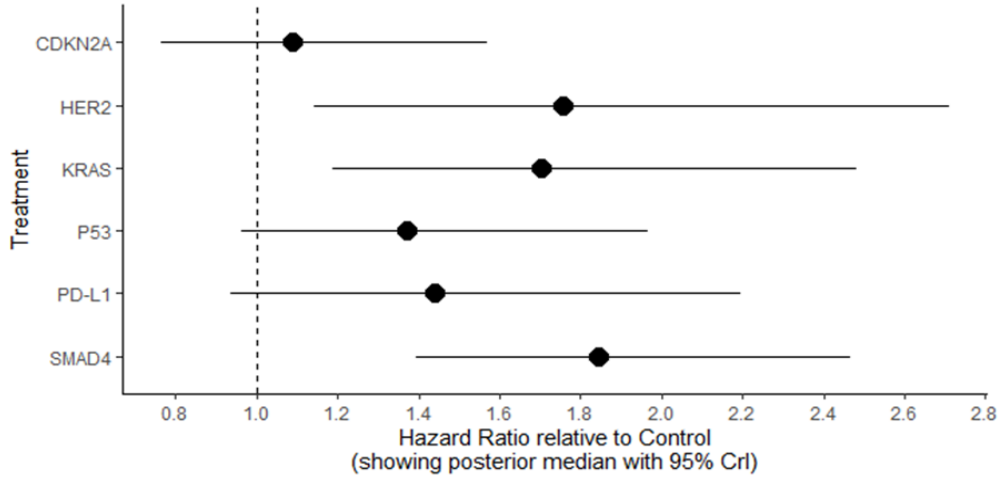

Figure 6. Forest Plot of This Network-Meta Analysis.

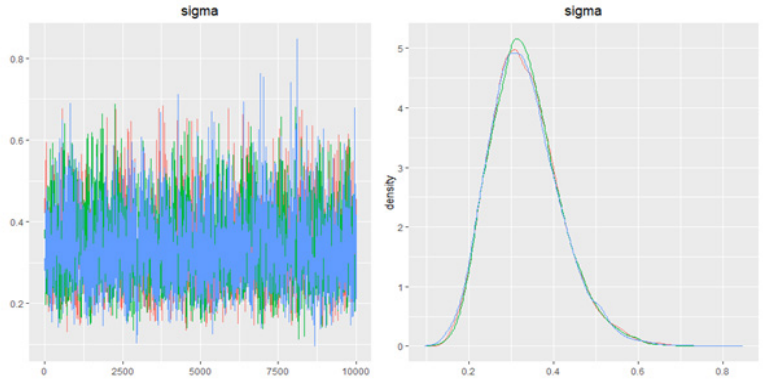

Figure 7. Convergence Model in This Network Meta-Analysis

use the same method to examine the gene mutations. Thus we cannot exclude the possibility that inconsistency exists in some of the included networks because the power of tests of inconsistency is limited. It is not known how such dependencies between NMAs might have affected the relationship between the contributions of different paths and the size and structure of the network. Thus, readers should take into account that the same studies might have been included in different NMAs when interpreting our results. Nevertheless, this was the first NMA that boardly analysis genetic mutations that contributed to the prognosis overall survival in pancreatic cacner.

In conclusion, in pancreatic cancer, the mutation of SMAD4 predicted the worst overall survival, compared to control, mutation of HER2, KRAS, PD-L1, P53, and CDKN2A.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

The authors acknowledged the Digestive Surgery Division which has indirectly contributed to the success of this study.

Conflict of interest

The authors declared that there wa no conflict of interest in this study.

References

Abramson MA, Jazag A, van der Zee JA,Whang EE (2007). The molecular biology of pancreatic cancer. Gastrointest *Cancer Res*, **1**, 7-12.

Austin PC, Wagner P, Merlo J (2017). The median hazard ratio: a useful measure of variance and general contextual effects in multilevel survival analysis. *Stat Med*, **36**, 928-38.

Bachet JB, Maréchal R, Demetter P, et al (2012). Contribution of CXCR4 and SMAD4 in predicting disease progression pattern and benefit from adjuvant chemotherapy in resected pancreatic adenocarcinoma. *Ann Oncol*, **23**, 2327-35.

Bailey P, Chang DK, Nones K, et al (2016). Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature*, **531**, 47-52.

Bamford S, Dawson E, Forbes S, et al (2004). The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. *Br J Cancer*, **91**, 355-8.

Barcot O, Boric M, Poklepovic Pericic T, et al (2019). Risk of bias judgments for random sequence generation in Cochrane systematic reviews were frequently not in line with Cochrane Handbook. *BMC Med Res Methodol*, **19**, 170.

Béliveau A, Boyne DJ, Slater J, Brenner D, Arora P (2019). BUGSnet: an R package to facilitate the conduct and reporting of Bayesian network Meta-analyses. *BMC Med Res Methodol*, **19**, 196.

Birnbaum DJ, Finetti P, Lopresti A, et al (2016). Prognostic value of PDL1 expression in pancreatic cancer. *Oncotarget*, **7**, 71198-210.

Blackford A, Serrano OK, Wolfgang CL, et al (2009). SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer. *Clin Cancer Res*, **15**, 4674-9.

Carrato A, Falcone A, Ducreux M, et al (2015). A Systematic Review of the Burden of Pancreatic Cancer in Europe: Real-World Impact on Survival, Quality of Life and Costs. *J Gastrointest Cancer*, **46**, 201-11.

Chen J, Li D, Killary AM, et al (2009). Polymorphisms of p16, p27, p73, and MDM2 modulate response and survival of pancreatic cancer patients treated with preoperative chemoradiation. *Ann Surg Oncol*, **16**, 431-9.

Cicenas J, Kvederaviciute K, Meskinyte I, et al (2017). KRAS, TP53, CDKN2A, SMAD4, BRCA1, and BRCA2 Mutations in Pancreatic Cancer. *Cancers (Basel)*, **9**.

De Bosscher K, Hill CS, Nicolás FJ (2004). Molecular and functional consequences of Smad4 C-terminal missense mutations in colorectal tumour cells. *Biochem J*, **379**, 209-16.

Grant TJ, Hua K, Singh A (2016). Molecular Pathogenesis of Pancreatic Cancer. *Prog Mol Biol Transl Sci*, **144**, 241-75.

Han SH, Ryu KH, Kwon AY (2021). The Prognostic Impact of HER2 Genetic and Protein Expression in Pancreatic Carcinoma-HER2 Protein and Gene in Pancreatic Cancer. *Diagnostics (Basel)*, **11**.

Hu H-f, Ye Z, Qin Y, et al (2021). Mutations in key driver genes of pancreatic cancer: molecularly targeted therapies and other clinical implications. *Acta Pharmacol Sin*, **42**, 1725-41.

Hua Z, Zhang YC, Hu XM,Jia ZG (2003). Loss of DPC4 expression and its correlation with clinicopathological parameters in pancreatic carcinoma. *World J Gastroenterol*, **9**, 2764-7.

Idachaba S, Dada O, Abimbola O, et al (2019). A Review of Pancreatic Cancer: Epidemiology, Genetics, Screening, and Management. *Open Access Maced J Med Sci*, **7**, 663-71.

Iwatate Y, Hoshino I, Ishige F, et al (2020). Prognostic significance of p16 protein in pancreatic ductal adenocarcinoma. *Mol Clin Oncol*, **13**, 83-91.

Jiang H, He C, Geng S, et al (2012). RhoT1 and Smad4 are correlated with lymph node metastasis and overall survival in pancreatic cancer. *PLoS One*, **7**, e42234.

Karamitopoulou E, Andreou A, Pahud de Mortanges A, et al (2021). PD-1/PD-L1-Associated Immunoarchitectural Patterns Stratify Pancreatic Cancer Patients into Prognostic/ Predictive Subgroups. *Cancer Immunol Res*, **9**, 1439-50.

Kinugasa H, Nouso K, Miyahara K, et al (2015). Detection of K-ras gene mutation by liquid biopsy in patients with pancreatic cancer. *Cancer*, **121**, 2271-80.

Komoto M, Nakata B, Amano R, et al (2009). HER2 overexpression correlates with survival after curative resection of pancreatic cancer. *Cancer Sci*, **100**, 1243-7.

Liang X, Sun J, Wu H, et al (2018). PD-L1 in pancreatic ductal adenocarcinoma: a retrospective analysis of 373 Chinese patients using an in vitro diagnostic assay. *Diagn Pathol*, **13**, 5.

Lin JC, Liu TP,Yang PM (2020). CDKN2A-Inactivated Pancreatic Ductal Adenocarcinoma Exhibits Therapeutic Sensitivity to Paclitaxel: A Bioinformatics Study. *J Clin Med*, **9**,

McIntyre CA, Lawrence SA, Richards AL, et al (2020). Alterations in driver genes are predictive of survival in patients with resected pancreatic ductal adenocarcinoma. *Cancer*, **126**, 3939-49.

Mizrahi JD, Surana R, Valle JW, Shroff RT (2020). Pancreatic cancer. *Lancet*, **395**, 2008-20.

Moher D, Shamseer L, Clarke M, et al (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev*, **4**, 1.

Oshima M, Okano K, Muraki S, et al (2013). Immunohistochemically detected expression of 3 major genes (CDKN2A/p16, TP53, and SMAD4/DPC4) strongly predicts survival in patients with resectable pancreatic cancer. *Ann Surg*, **258**, 336-46.

Ottenhof NA, Morsink FHM, Ten Kate F, van Noorden CJF, Offerhaus GJA (2012). Multivariate analysis of immunohistochemical evaluation of protein expression in pancreatic ductal adenocarcinoma reveals prognostic significance for persistent Smad4 expression only. *Cell Oncol*, **35**, 119-26.

Principe DR, Underwood PW, Kumar S, et al (2022). Loss of SMAD4 Is Associated With Poor Tumor Immunogenicity and Reduced PD-L1 Expression in Pancreatic Cancer. *Front Oncol*, **12**, 806963.

Rawla P, Sunkara T,Gaduputi V (2019). Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors. *World J Oncol*, **10**, 10-27.

Rice A, Del Rio Hernandez A (2019). The Mutational Landscape of Pancreatic and Liver Cancers, as Represented by Circulating Tumor DNA. *Front Oncol*, **9**, 952.

Salanti G, Ades AE Ioannidis JP (2011). Graphical methods and numerical summaries for presenting results from multiple-treatment meta-analysis: an overview and tutorial. *J Clin Epidemiol*, **64**, 163-71.

Saxby AJ, Nielsen A, Scarlett CJ, et al (2005). Assessment of HER-2 status in pancreatic adenocarcinoma: correlation of immunohistochemistry, quantitative real-time RT-PCR, and FISH with aneuploidy and survival. *Am J Surg Pathol*, **29**, 1125-34.

Schultheis B, Reuter D, Ebert MP, et al (2017). Gemcitabine combined with the monoclonal antibody nimotuzumab is an active first-line regimen in KRAS wildtype patients with locally advanced or metastatic pancreatic cancer: a multicenter, randomized phase IIb study. *Ann Oncol*, **28**, 2429-35.

Sharif S, Ramanathan RK, Potter D, Cieply K, Krasinskas AM (2008). HER2 gene amplification and chromosome 17 copy number do not predict survival of patients with resected pancreatic adenocarcinoma. *Dig Dis Sci*, **53**, 3026-32.

Shen H, Lundy J, Strickland AH, et al (2022). KRAS G12D Mutation Subtype in Pancreatic Ductal Adenocarcinoma: Does It Influence Prognosis or Stage of Disease at Presentation?. *Cells*, **11**.

Shibata W, Kinoshita H, Hikiba Y, et al (2018). Overexpression of HER2 in the pancreas promotes development of intraductal papillary mucinous neoplasms in mice. *Sci Rep*, **8**, 6150.

Shin SH, Kim HJ, Hwang DW, et al (2017). The DPC4/SMAD4 genetic status determines recurrence patterns and treatment outcomes in resected pancreatic ductal adenocarcinoma: A prospective cohort study. *Oncotarget*, **8**, 17945-59.

Shin SH, Kim SC, Hong SM, et al (2013). Genetic alterations of K-ras, p53, c-erbB-2, and DPC4 in pancreatic ductal adenocarcinoma and their correlation with patient survival. *Pancreas*, **42**, 216-22.

Sung H, Ferlay J, Siegel RL, et al (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*, **71**, 209-49.

Voutsadakis IA (2021). Mutations of p53 associated with pancreatic cancer and therapeutic implications. *Ann Hepatobiliary Pancreat Surg*, **25**, 315-27.

Waddell N, Pajic M, Patch AM, et al (2015). Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature*, **518**, 495-501.

Wang L, Ma Q, Chen X, et al (2010). Clinical significance of B7-H1 and B7-1 expressions in pancreatic carcinoma. *World J Surg*, **34**, 1059-65.

Wang Y, Lin J, Cui J, et al (2017). Prognostic value and clinicopathological features of PD-1/PD-L1 expression with mismatch repair status and desmoplastic stroma in Chinese patients with pancreatic cancer. *Oncotarget*, **8**, 9354-65.

Waters AM, Der CJ (2018). KRAS: The Critical Driver and Therapeutic Target for Pancreatic Cancer. *Cold Spring Harb Perspect Med*, **8**,

Windon AL, Loaiza-Bonilla A, Jensen CE, et al (2018). A KRAS wild type mutational status confers a survival advantage in pancreatic ductal adenocarcinoma. *J Gastrointest Oncol*, **9**, 1-10.

Yamaki S, Yanagimoto H, Tsuta K, Ryota H, Kon M (2017). PD-L1 expression in pancreatic ductal adenocarcinoma is a poor prognostic factor in patients with high CD8(+) tumor-infiltrating lymphocytes: highly sensitive detection using phosphor-integrated dot staining. *Int J Clin Oncol*, **22**, 726-33

Zhao L, Cao Y (2020). PD-L1 Expression Level Displays a Positive Correlation with Immune Response in Pancreatic Cancer. *Dis Markers*, **2020**, 8843146.