Molecular Characterization and Clinical Outcomes of Pancreatic Neuroendocrine Neoplasms Harboring PAK4-NAMPT Alterations

Ibrahim Azar, MD[1,2]; Husain Yar Khan, PhD[2,3]; Sahar F. Bannoura, MSc[3]; Nishant Gandhi, PhD[4]; Md Hafiz Uddin, PhD[3]; Misako Nagasaka, MD[5]; Jun Gong, MD[4]; Bassel Nazha, MD, MPH[6]; Khalil Choucair, MD, MSc[3]; Nikhil Vojjala, MD[1]; Moh'd M. Khushman, MD[7]; Heloisa P. Soares, MD[8]; Wafik S. El-Deiry, MD, PhD, FACP[9]; Philip Agop Philip, MD, PhD, FRCP[10]; Bassel El-Rayes, MD[11]; Herbert Chen, MD, FACS[11]; Emil Lou, MD, PhD[12]; Irfana Muqbil, PhD[13]; Alex Patrick Farrell, PhD[4]; Jeffrey Swensen, PhD[4]; Matthew James Oberley, MD, PhD[4]; Chadi Nabhan, MD, MBA, FACP[4]; Sanjay Goel, MD, MS[4]; Anthony F. Shields, MD[3]; Ramzi M. Mohammad, PhD[3]; Boris C. Pasche, MD, PhD, FACP[3]; and Asfar S. Azmi, PhD[3]

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ABSTRACT

PURPOSE The mammalian target of rapamycin (mTOR) inhibitor everolimus is US Food and Drug Administration-approved for advanced pancreatic neuroendocrine neoplasms (pNENs), yet resistance is common, necessitating the identification of resistance mechanisms for effective treatment strategies. Previous studies suggest that targeting the aberrant expression of mTOR regulators p21-activated kinase 4 (PAK4) and nicotinamide phosphoribosyl transferase (NAMPT) sensitizes pNENs to everolimus. In this study, we queried a large real-world data set of pNENs, characterizing the molecular and immune landscapes, as well as the clinical outcomes associated with aberrant PAK4 and NAMPT expression.

METHODS Two-hundred and ninety-four pNEN cases were analyzed using next-generation sequencing and whole-exome/whole-transcriptome sequencing. We stratified patients into clusters on the basis of median cutoff.

RESULTS High expression of genes activated in response to mTOR activation was found in NAMPT-high and PAK4-high groups. Enrichment of PI3K/AKT/mTOR and glycolysis pathways was observed in these tumors. Higher mutation rates in multiple endocrine neoplasia type 1, alpha thalassemia/mental retardation syndrome X-linked, TSC2, SETD2, and CCNE1 were observed in high NAMPT and PAK4 clusters. Immune analysis revealed enrichment in inflammatory response pathways, IL2/STAT5 signaling, and immune checkpoint genes. Increased neutrophils, natural killer cells, and macrophages were found in PAK4-high/NAMPT-high tumors. Analysis of real-world patient data revealed that high PAK4 ($P = .0428$) or NAMPT ($P = .0002$) expression individually correlated with lower overall survival in all neuroendocrine neoplasms (NEN) cohorts, while the combined high expression of both was associated with the worst outcomes ($P = .0002$). Similar trends were observed in pancreatic NEN cohorts.

CONCLUSION Our study demonstrates that PAK4-high/NAMPT-high pNENs are associated with distinct molecular and immune profiles. Further investigation is warranted to determine if dual PAK4 and NAMPT blockade enhances the efficacy of immunotherapeutics.

ACCOMPANYING CONTENT

Appendix

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INTRODUCTION

According to the American Cancer Society's estimate, over 4,300 individuals will be diagnosed with pancreatic neuroendocrine neoplasms (pNENs) in 2023 (SEER Statistics).[1] The survival for pNENs is relatively long, which makes the prevalence of this disease high at any given time. Patients with well and moderately differentiated pNENs can remain asymptomatic for many years and present only at an advanced stage. The management of pNENs remains clinically challenging due in part to the heterogeneity of the disease.[2] Over the past decades, improvements in the identification of actionable molecular targets or systemic treatments for pNENs have been modest at best. Therefore, pNENs remain a significant unmet clinical problem in urgent need of newer therapeutics.

CONTEXT

Key Objective

We aimed to characterize the molecular and immune landscapes of pancreatic neuroendocrine neoplasms (pNENs) with aberrant p21-activated kinase 4 (PAK4) and nicotinamide phosphoribosyl transferase (NAMPT) expression and delineate their impact on clinical outcomes.

Knowledge Generated

We identified three distinct clusters on the basis of PAK4/NAMPT expression. We found an association of high PAK4/NAMPT expression with mammalian target of rapamycin activation and glycolytic pathway enrichment, increased mutations in specific genes, and altered immune profiles. High PAK4 or NAMPT expression individually correlated with lower overall survival, and combined high expression resulted in the worst outcomes. These results suggest potential therapeutic strategies for pNENs, especially for those resistant to everolimus.

Relevance *(A.R. Parikh)*

Everolimus is a well-established therapeutic option for pancreatic neuroendocrine tumors (pNETs) but resistance is common. The authors evaluate the role of PAK4-NAMPT expression for in this pNET and showed distinct molecular and immune profiles in tumors with high expression but also worse outcomes that may lead to future therapeutic strategies.*

Plain Language Summary *(M. Lewis)*

Everolimus is a well-established therapeutic option for pNETs, but resistance is common. The authors evaluate the role of PAK4-NAMPT expression for pNETs and showed distinct molecular and immune profiles in tumors with high expression of PAK4-NAMPT, but also with this marker, the authors showed worse outcomes and important as targeting this pathway that may lead to future therapies.†

*Relevance section written by *JCO Oncology Advances* Associate Editor Aparna R. Parikh, MD.
†Plain Language Summary written by *JCO Oncology Advances* Associate Editor Mark Lewis, MD.

Besides loss of multiple endocrine neoplasia type 1 (MEN1), death domain–associated protein, and alpha thalassemia/mental retardation syndrome X-linked (ATRX) genes, the hyperactivation of PI3K/Akt/mammalian target of rapamycin (mTOR) through loss of tumor suppressor PTEN has been well documented as one of the main drivers in pNENs.[3] This was the basis for targeting the mTOR pathway. Furthermore, the mTOR inhibitor everolimus is approved by the US Food and Drug Administration (FDA) for patients with pNENs and shows an improvement in median overall survival of 11.1 months compared with 4.4 months with a placebo.[4] Nevertheless, primary or acquired resistance to mTOR inhibitors is observed in the majority of patients.[5] There are several mechanisms of everolimus resistance, including incomplete inhibition of the mTOR pathway, as everolimus is only specific toward mTORC1 and not mTORC2 in the mTOR complex.[5]

p21-activated kinase 4 (PAK4) is a group II PAK family member that controls invadopodia and migratory behavior.[6] PAK4 is a node protein linking different MAPK downstream pathways.[7] PAK4 regulates several substrates in the PI3K/AKT/mTOR signaling pathway through phosphorylation that regulates the pathogenesis and evolution of pNENs.[8] Studies show that PAK4 is a key effector of the Rho GTPases that directly regulates mTORC2 and its downstream effectors (such as pS6K, 4EBP1, and eIF4E), thereby promoting everolimus resistance.[9] In this direction, it was shown earlier that PAK4 is aberrantly overexpressed in pNENs and that RNAi knockdown or chemical inhibition of PAK4 resensitizes pNENs to everolimus and other pNEN therapies.[10] Thus, PAK4 cross-talks with pNEN driver pathways, making this kinase an attractive target for this treatment-refractory disease.

Deregulated metabolism is a hallmark of all cancers, and NENs are no exception. Tumor cells rely on high glycolysis, pentose, and fatty acid biosynthesis.[11] Deregulated tumor energetics result in tumors turning to alternative energy sources, especially the nicotinamide adenine dinucleotide (NAD) pool.[12] Studies have shown that the NAD biosynthetic pathway is deregulated in pNENs, making it an attractive therapeutic target.[13] Nicotinamide phosphoribosyl transferase (NAMPT) is the rate-limiting enzyme in the NAD salvage pathway, which is one of the major sources of NAD in eukaryotes.[14] In neuroendocrine tumors, NAMPT has been identified as a mechanistic dependency and an attractive therapeutic target.[15] Given the significance of PAK4 and NAMPT in the biology of NENs, in this study, we queried the importance of the expression of these genes in a large real-world data set of pNENs. Our goals were to study the association between PAK4/NAMPT and clinical outcomes, especially in patients treated with everolimus, and to

characterize the molecular and immune landscapes associated with PAK4/NAMPT expression.

METHODS

Tissue Samples

pNEN specimens were sequenced for genomic (DNA-592 gene panel/whole-exome sequencing) and transcriptomic (whole-transcriptomic sequencing) analyses by Caris Life Sciences (Phoenix, AZ) as part of routine comprehensive molecular profiling. Before molecular testing, tumor enrichment was achieved by harvesting targeted tissue using manual microdissection techniques.

Next Generation Sequencing

Genomic DNA was isolated from formalin-fixed paraffin-embedded (FFPE) samples that underwent microdissection to enrich tumor purity and subjected to next-generation sequencing (NGS) using the NextSeq or NovaSeq 6000 platforms (Illumina, Inc, San Diego, CA). Details of the methods and list of genes assayed can be found elsewhere.[16] The copy-number alteration of each exon was determined by calculating the average depth of the sample along with the sequencing depth of each exon and comparing this calculated result with a precalibrated value, and a copy number of ≥6.0 was considered amplified. Tumor mutational burden (TMB) was measured by counting all nonsynonymous missense, nonsense, in-frame insertion/deletion, and frameshift mutations found per tumor that had not been previously described as germline alterations in dbSNP151, Genome Aggregation Database databases, or benign variants identified by Caris's geneticists. TMB-H was defined as ≥10 mutations per megabase. For samples profiled by whole exome sequencing, genomic loss of heterozygosity (LOH) was calculated by dividing 22 autosomal chromosomes into 552 segments and calculating the LOH of single nucleotide polymorphisms within each segment, with 99% of segments at least 5 Mb in length and segments spanning ≥90% of a whole chromosome or chromosome arm excluded from the calculation. NGS of RNA on FFPE samples was performed using the Agilent SureSelect Human All Exon V7 bait panel (Agilent Technologies, Santa Clara, CA) and the Illumina NovaSeq platform (Illumina, Inc, San Diego, CA) as described previously.[17] Single-sample gene set enrichment analysis (ssGSEA) was performed for hallmark analysis using previously published methods.[18,19]

Immunohistochemistry

For PD-L1+ expression, multiple antibody clones were used. The antibody clone 22c3 (Dako, Santa Clara, CA) was measured using the tumor proportion score, which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity, with a positive threshold of ≥1%. For the 28-8 antibody clone, PD-L1 expression on tumor cells was scored using an FDA-approved test (pharmDx, Santa Clara, CA) and a positive threshold of ≥1+ stain intensity and ≥1% cells stained. For the SP142 antibody clones, PD-L1 expression was scored on tumor cells using a laboratory-developed test and a positive threshold of ≥2+ stain intensity and ≥5% cells stained. By contrast, PD-L1 expression on immune cells was scored using an FDA-approved test (Ventana; Roche, Indianapolis, IN) and a positive threshold of ≥1+ stain intensity and ≥10% cells stained.

Gene Fusion Detection

Gene fusions were detected by RNA sequencing using either the ArcherDx fusion assay (Archer FusionPlex Solid Tumor panel) or whole-transcriptome sequencing assay (Illumina NovaSeq platform; Illumina, Inc, San Diego, CA). Variants of genes were predetermined for their cancer-related and clinical significance as described previously.[20]

Real-World Survival Analysis

Overall survival was calculated using a repository of real-world evidence (RWE) insurance claim data, with overall survival defined from the date of biopsy or start of therapy until last contact. Patients who did not have an observed claim within 100 days of the end of available RWE records were presumed to be deceased and uncensored, which was found to be 95% concordant to data obtained from National Death Index data (National Center for Health Statistics, Centers for Disease Control and Prevention). All other patients were censored. Hazard ratios for survival (and 95% CIs) were computed using the Cox proportional hazards model, and overall survival between groups was compared using the log-rank test.

Statistical Analysis

Molecular associations were tested by using chi-square and Fisher's exact tests for categorical variables and Mann-Whitney *U* for continuous variables, where appropriate. Statistical analyses were performed with open-source Python libraries.

Ethics Statement

This study was conducted in accordance with guidelines of the Declaration of Helsinki, Belmont Report, and US Common Rule. In keeping with 45 CFR 46.101(b) (4), this study was performed using retrospective, deidentified clinical data. Therefore, this study was considered IRB-exempt and no patient consent was necessary from the patients.

RESULTS

Association of PAK4 and NAMPT Expression With mTOR and Glycolytic Pathway Genes

Hierarchical agglomerative clustering on the expression of PAK4 and NAMPT was used to stratify 294 cases of pNENs

TABLE 1. Baseline Characteristics of the Study Population

Cohort Characteristic	NAMPT-High Cluster (n = 127)	PAK4-High Cluster (n = 138)	NAMPT PAK4-Low Cluster (n = 29)	Statistic Used	*P*	q-value
Median age, years (range) (No.)	59 (20-85) (127)	65 (20 to >89) (138)	59 (32-77) (29)	Kruskal-Wallis test	.00025	0.0005
Males	57.5% (73/127)	60.1% (83/138)	55.2% (16/29)	Chi-square test	.843	0.843
Females	42.5% (54/127)	39.9% (55/138)	44.8% (13/29)	Chi-square test	.843	0.843
Smoker	100% (5/5)	100% (13/13)	100% (5/5)	No test performed	NaN	NaN

Abbreviations: NAMPT, nicotinamide phosphoribosyl transferase; NaN, not a number; PAK4, p21-activated kinase 4.

into three clusters: PAK4-low-NAMPT-low (29 cases), PAK4-high (138 cases), and NAMPT-high (127 cases). PAK4-high cluster was associated with tumors biopsied from patients who were older (median age 65 years) compared with those with tumors within NAMPT-high (median age 59 years) or PAK4-low-NAMPT-low (median age 59 years) clusters. The distribution of sex was similar between the three groups and patients were identified as smokers by history (Table 1).

We queried the expression of a panel of genes activated in response to mTOR activation and found that these genes, including Glut1 promoter solute carrier family 2 member 1 (*SLC2A1*; 2.06 to 3.36-fold), glucose regulator phosphofructokinase (*PFKP*; 2.14 to 2.86-fold), monosaturated fatty acid synthesis enzyme stearoyl-CoA desaturase (*SCD*; 2.75 to 3.97-fold), mevalonate kinase (*MVK*; 2.29 to 3.48-fold), and glucose 6 phosphate dehydrogenase (*G6PD*; 1.75 to 2.63-fold), were all overexpressed in NAMPT-high or PAK4-high compared with the PAK4-low/NAMPT-low tumors (all $q < 0.05$; Figs 1A and 1B). However, the ssGSEA did not reveal a significant enrichment in the PI3K/AKT/mTOR and mTORC1 signaling pathways within the tumors displaying high levels of PAK4 and NAMPT (Appendix Fig A1). Notably, our analysis highlighted that the deviations in mTOR pathway genes were most pronounced in the PAK4-high/NAMPT-high group compared with other groups. Furthermore, the observed enrichment in pathways related to inflammatory responses, such as tumor necrosis factor-α, and IL2/STAT5 signaling pathways (Fig 2A), suggests a significant involvement of these immune and inflammatory pathways in the tumor microenvironment in the NAMPT-high cluster. Although weak, the IL2/STAT5 and inflammatory pathways were positively correlated with NAMPT expression. No such correlation was observed with PAK4 expression (Fig 2B). This enrichment might indicate a proinflammatory environment within these tumors, potentially influencing their progression and response to therapeutic interventions.

Comutations Associated With PAK4 and NAMPT Expression

We next analyzed the co-occurrence of mutations of prominent genes in the mTOR pathway in relation to PAK4 and NAMPT expression in the pNEN cohort under study. Although not statistically significant, we observed higher rates of mutations in MEN1, ATRX, TSC2, SETD2, and CCNE1 in NAMPT-high and PAK4-high clusters (Fig 3A). Interestingly, mutations in TSC3, SETD2, and CCNE2 were not observed in the PAK4-low-NAMPT-low group, while ATM mutations were only observed in the NAMPT-high cluster (Fig 3A). However, RB1 mutations were higher in PAK4-high cluster in comparison with PAK4-low-NAMPT-low cluster. RB1 mutations were less in the NAMPT-high cluster compared with PAK4-low-NAMPT-low group. More intriguingly, p53 mutations were less prevalent in the PAK4-high or NAMPT-high clusters versus PAK4-low-NAMPT-low groups (Fig 3B).

Association of PAK4 and NAMPT Expression With Immune Checkpoint Gene Expression and Immune Cell Infiltration

In pNENs characterized by heightened levels of NAMPT or PAK4, a significant enrichment in the expression of immune checkpoint genes was observed compared with the PAK4-low-NAMPT-low group. Specifically, genes such as LAG3 (2.61 to 3.19-fold), CD80 (2.93 to 2.78-fold), IDO1 (3.43 to 2.2-fold), CD274 (3.75 to 3.62-fold), CD86 (4.28 to 3.37-fold), PDCD1LG2 (3.68 to 2.86-fold), and HAVCR2 (4.09 to 3.99-fold; all $q < 0.05$) exhibited notably elevated expression levels (Fig 4). A moderate positive correlation was observed between the expressions of HAVCR2 and CD86 and NAMPT (Appendix Fig A2).

Furthermore, compared with PAK4-low-NAMPT-low tumors, an enrichment in B cells, M1 and M2 macrophages, and neutrophils was observed in tumors belonging to the NAMPT-high cluster (all $q < 0.05$). A similar enrichment in M2 macrophages, neutrophils, and natural killer cells was also observed in the PAK4-high compared with the PAK4-low-NAMPT-low cluster (all $q < 0.05$). No significant differences were observed in CD4+ and CD8+ T cells among the three groups (Fig 5).

Association of PAK4 and NAMPT Expression With Prognosis of NENs

Kaplan-Meier curves showed an association of PAK4 and NAMPT expression with real-world survival data from all

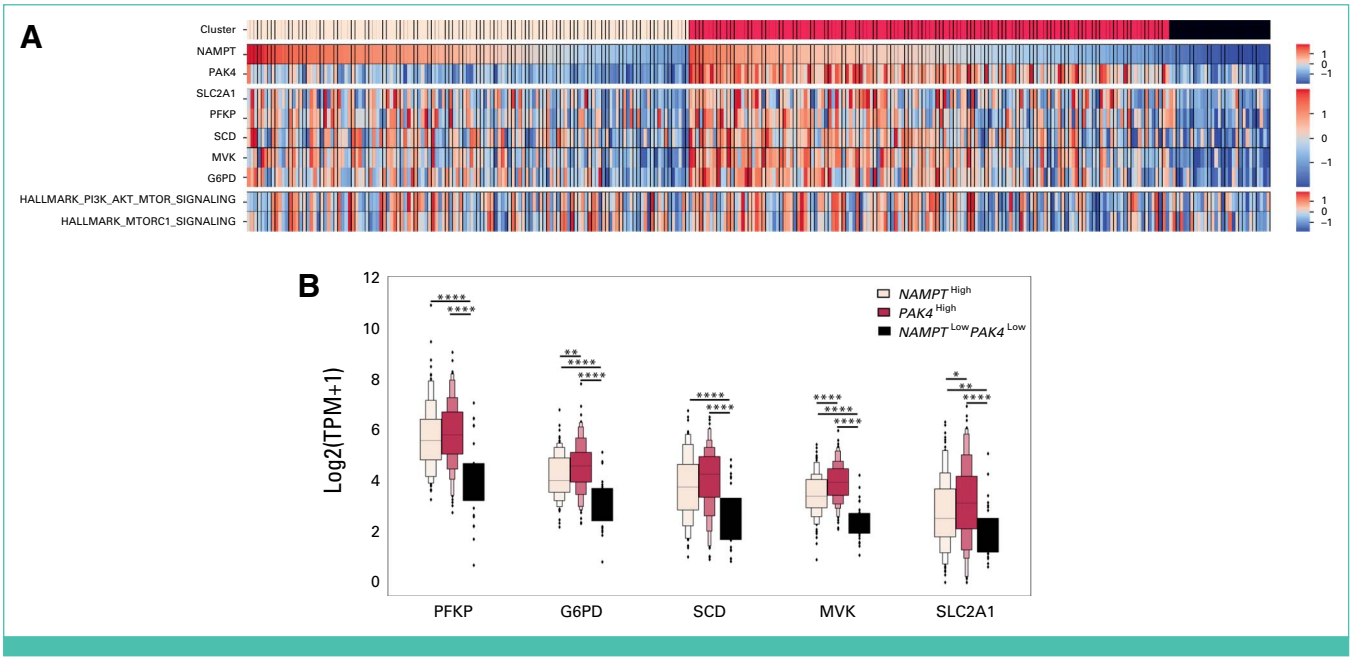

FIG 1. (A) The heat map of the various clusters in the study on the basis of the molecular profiling of pNENs on the basis of PAK4 and NAMPT expression. The entire population is divided into three clusters. NAMPT-high, PAK4-high, and NAMPT, PAK4-low. (B) The box and whisker plots of the panel of genes activated in response to mTOR activation. SLC2A1, PFKP, SCD, MVK, and G6PD were found to be all overexpressed in NAMPT-high or PAK4-high compared with low tumors (all q < 0.05). G6PD, glucose 6 phosphate dehydrogenase; mTOR, mammalian target of rapamycin; MVK, mevalonate kinase; NAMPT, nicotinamide phosphoribosyl transferase; pNENs, pancreatic neuroendocrine neoplasms; PAK4, p21-activated kinase 4; PFKP, phosphofructokinase; SCD, stearoyl-CoA desaturase; SLC2A1, solute carrier family 2 member 1.

neuroendocrine neoplasms (NEN) and pancreatic NEN patient cohorts (Appendix Fig A3). Patient cohorts were stratified by PAK4 (high *v* low) and NAMPT (high *v* low) median expression. Patient cohorts with either high PAK4 or high NAMPT had lower overall survival compared with low PAK4 or low NAMPT cohorts, respectively (Appendix Figs A3A and A3B). In addition, patients with both high PAK4 and high NAMPT had worse survival outcomes compared with PAK4-low and NAMPT-low tumors (Appendix Fig A3C). A subgroup analysis showed no significant difference in survival on everolimus, potentially because of low number of cases. Although not statistically significant, similar survival trends were observed when the pancreatic NEN patient cohorts were stratified on the same lines as mentioned above (Appendix Figs A3D-A3H).

Dual Targeting of PAK4 and NAMPT Decreases Viability of pNEN Cells

Our previous studies have shown that the dual inhibition of PAK4 and NAMPT either by siRNA or small molecule KPT-9274 inhibits pNEN cellular and in vivo xenograft models.[10] Here, we overexpressed PAK4 in BON-1 pNEN cells (Appendix Fig A4A). PAK4 and NAMPT dual inhibition resulted in significant loss of cell viability, which was rescued by PAK4 overexpression (OE; Appendix Fig A4B). Similarly, PAK4 OE reduced the response to everolimus (Appendix Fig A4C). OE of NAMPT or niacin treatment rescued the response to KPT-9274, indicating NAMPT-specific response to KPT-9274 (Appendix Figs A4D-A4F). Additionally, we evaluated the expression of PAK4 in pNEN tumors compared with matched normal control using immunohistochemistry staining, which showed higher intensity of staining in tumor tissues (Appendix Fig A5) Overall, these data show the dependency of pNEN cells on PAK4 and NAMPT for their survival and growth.

DISCUSSION

In this article, we characterize the impact of high PAK4 and NAMPT on pNEN mutation, metabolic, and immune landscapes. Our findings offer extensive insights into the molecular and immune landscapes. The significance of these aberrations in pNENs have implications for the treatment strategies and potentially the efficacy of existing therapies.

The hyperactivation of PI3K/Akt/mTOR through loss of tumor suppressor PTEN has been well documented as a main driver in pNENs. This led to the introduction of mTOR inhibitor everolimus for the treatment of low-grade pNENs.[4] Nevertheless, responses to everolimus have been modest at best and resistance to everolimus is universal.[21] There are several factors associated with resistance to mTOR inhibition[22] including incomplete inhibition of mTOR as well as compensatory reactivation of mTORC2 feeding forward the

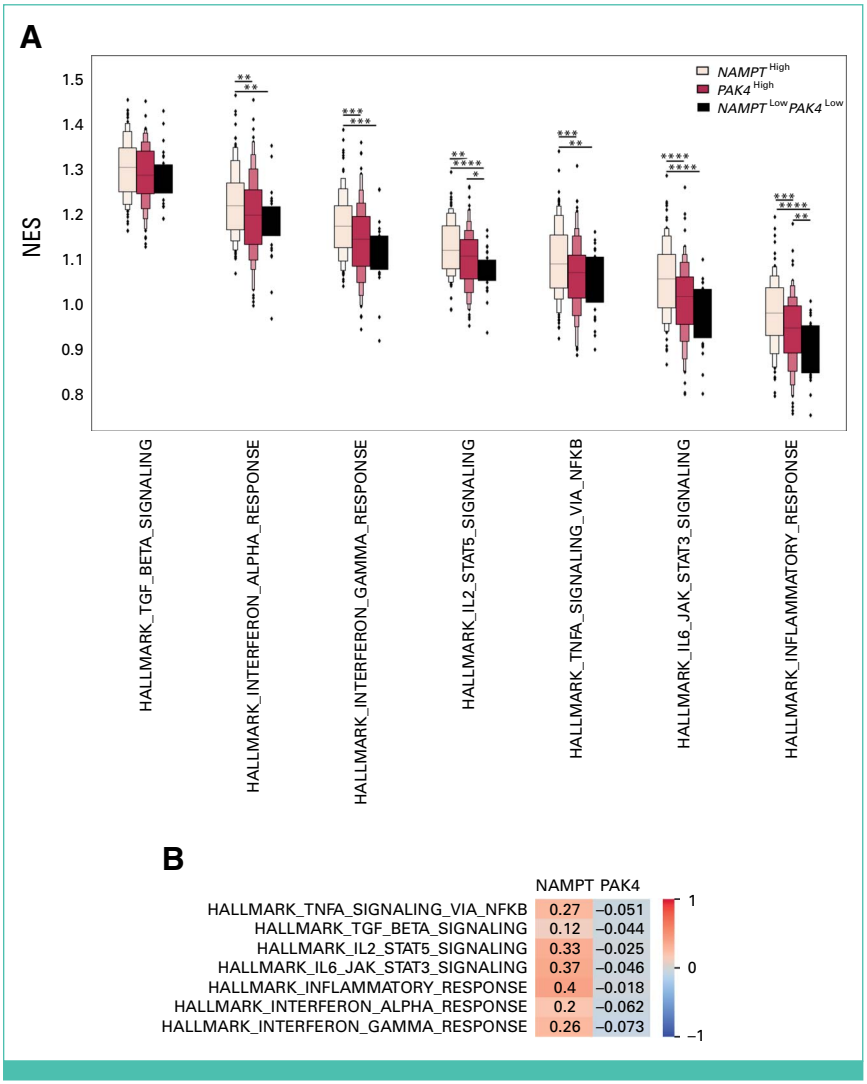

FIG 2. Box and whisker plots show enrichment analysis of inflammatory signaling pathways in pNENs. (A) TNF-α and IL2/STAT5 signaling pathways in the tumor microenvironment of the NAMPT-high cluster. (B) No such correlation of the inflammatory signals with PAK4 expression. NAMPT, nicotinamide phosphoribosyl transferase; NES, normalized enrichment score; PAK4, p21-activated kinase 4; pNENs, pancreatic neuroendocrine neoplasms; TNF, tumor necrosis factor.

mTOR signaling.[23] Therefore, identification of the mechanisms of mTOR inhibition resistance is of utmost importance to design strategies that could enhance the response to everolimus in patients with pNENs.

Earlier studies have shown that Rho GTPase effectors such as RAC can regulate mTORC1 and mTORC2.[24] PAK4 is a key effector of the Rho GTPases that directly regulate several mTORC2 downstream effectors (such as pS6K, 4EBP1, and eIF4E), thereby promoting everolimus resistance.[25] PAK4 is an effector of the Rho GTPases signaling pathway[26] and is known as a core molecule linking Ras/Erk, Wnt/β-catenin, and androgen receptor−/estrogen receptor−dependent pathways.[27] Thus, PAK4 interacts with pNEN driver pathways, making this kinase an attractive target for resistant pNENs. We also showed that PAK4 is aberrantly overexpressed in pNENs and that RNAi knockdown or chemical inhibition of PAK4 resensitizes pNENs to everolimus and other pNEN therapies.[10,28]

Deregulated metabolism is a hallmark of cancer, including NENs and pNENs.[29] Several metabolites shed by neuroendocrine cells have been identified to engage in cellular signaling and have paracrine effects on tumor cell proliferation.[29] Deregulated tumor energetics also drive tumors to seek alternative energy sources, especially the NAD pool. NAD is produced through the de novo pathway using tryptophan.[12] However, tumors depend on other NAD-producing mechanisms such as Preiss-Handler and salvage pathways.[12] Nicotinate phosphoribosyltransferase (NAPRT) is the rate-

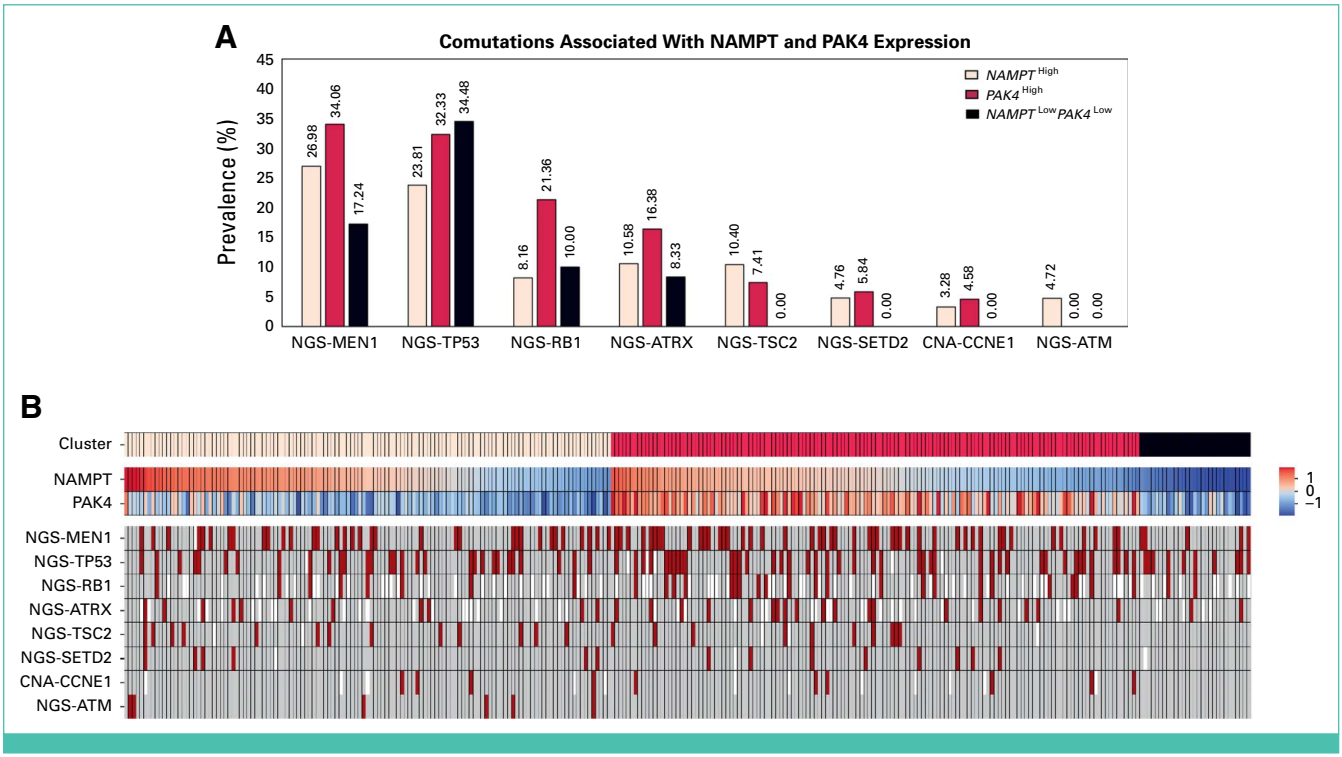

FIG 3. Comutations associated with PAK4 and NAMPT expression in pNENs. (A) Bar diagram showing higher rates of mutations in MEN1, ATRX, TSC2, SETD2, and CCNE1 in the NAMPT-high and PAK4-high clusters (P = >.05). (B) The heat map of the same. ATM mutations were identified only in NAMPT-high clusters, while RB1 was higher in the PAK4-high cluster. P53 mutations were less prevalent in the PAK4-high or NAMPT-high clusters versus low groups. NAMPT, nicotinamide phosphoribosyl transferase; PAK4, p21-activated kinase 4; pNENs, pancreatic neuroendocrine neoplasms.

limiting enzyme in the Preiss-Handler pathway that converts niacin to nicotinic acid mononucleotide (NAMN) that in turn is converted into nicotinic acid adenine dinucleotide before NAD production by NAD synthetase.[12] As a tumor metabolic liability, NAPRT is often lost in cancer due to hypermethylation[30]; thus, the salvage pathway is the major source of NAD synthesis by tumors, creating dependency on NAMPT. Our previous work has established that NAMPT is overexpressed in pNEN tissue and can be targeted using PAK4-NAMPT dual inhibitor.[10,28]

Notably, the current analysis revealed distinct profiles among the different groups stratified on the basis of PAK4 and NAMPT expression. The PAK4-high, NAMPT-high groups exhibited elevated expression of genes associated with mTOR activation, glycolysis, and pathways linked to PI3K/AKT/mTOR. These findings strongly suggest a convergence of the PAK4-mTOR-NAD axis within pNENs. The heightened activity in these pathways implies a potential strategy for effectively targeting these tumors. Specifically, the dual targeting of PAK4 and NAMPT could be an effective approach, particularly in sensitizing tumors that have shown resistance to everolimus. These observations suggest a robust association between PAK4/NAMPT expression and the dysregulation of these pivotal oncogenic pathways in pNENs.

Furthermore, this study sheds light on the immune landscape, revealing enrichment in various immune checkpoint genes and signaling pathways, such as those involved in the inflammatory response and IL2/STAT5 pathways, within the PAK4-high and NAMPT-high tumors. The increased expression of immune checkpoint genes and altered immune cell infiltration in NAMPT-high and PAK4-high tumors suggests potential immunotherapeutic vulnerabilities or opportunities for treatment intervention. It is notable that several recent studies have implicated PAK4 and NAMPT in immune surveillance.[31,32] For example, Tang and colleagues have shown using pan-cancer analysis the immunologic and prognostic role of PAK4.[33] Similarly, PAK4 has been suggested to regulate multiple aspects of immune biology and has been termed as an immune evasion target for cancer.[34] Several groups have demonstrated that PAK4 inhibition can enhance the antitumor activity of immune checkpoint blockade[35–37] and sensitize tumors to CAR-T therapy.[38]

Also, many investigations have pointed to the role of NAD metabolism in immune modulation.[39] High NAD+ metabolism has been shown to maintain inducible PD1 levels resulting in immune evasion.[40] Extracellular NAMPT has been shown to promote M2 macrophage polarization in hematologic malignancies.[41] NAMPT has also been shown to be critical for promoting tumor angiogenic activity of

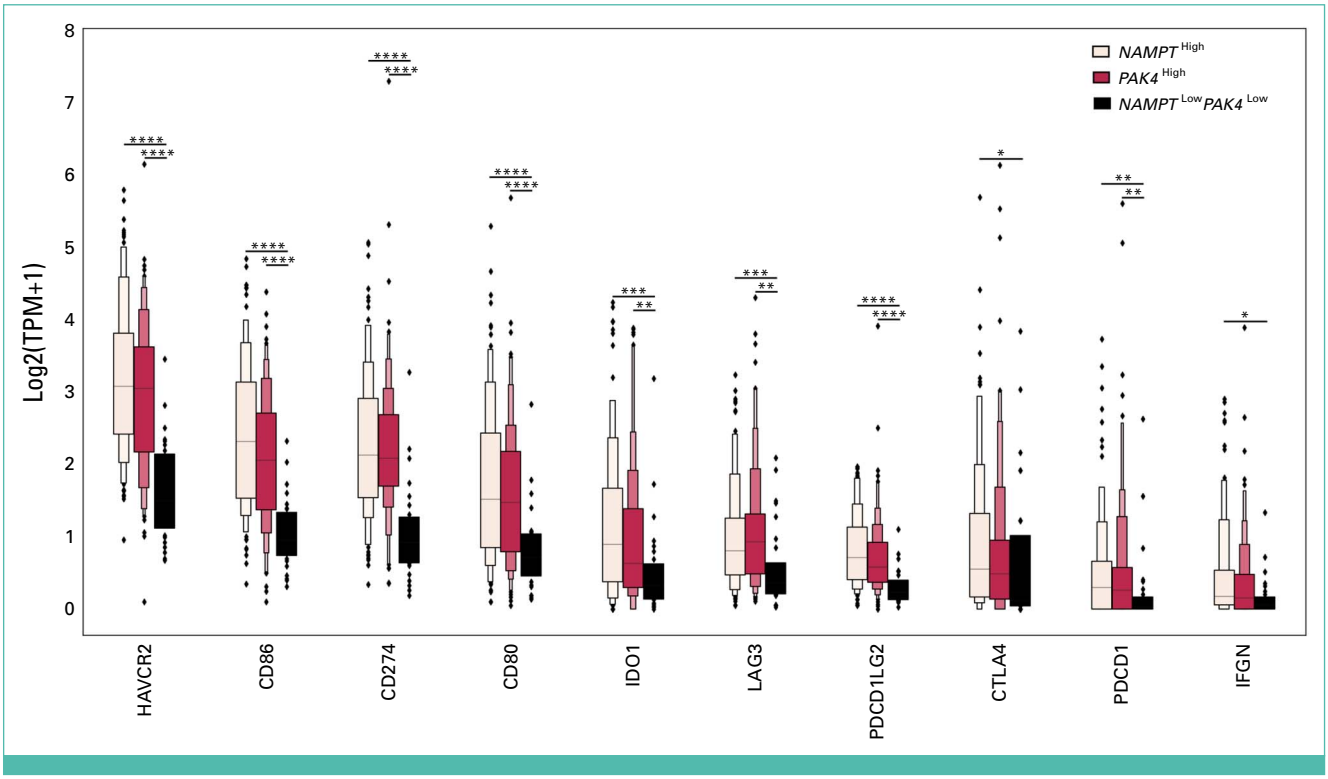

FIG 4. Box and whisker plots demonstrating the effect of PAK4 and NAMPT alterations on the expression of immune checkpoint genes in pNENs. Genes such as LAG3, CD80, IDO1, CD274, CD86, PDCD1LG2, and HAVCR2 were richly expressed in PAK4/NAMPT-high clusters compared with PAK4/NAMPT-low clusters and it is statistically significant ($q < 0.05$). NAMPT, nicotinamide phosphoribosyl transferase; PAK4, p21-activated kinase 4; pNENs, pancreatic neuroendocrine neoplasms.

neutrophils.[42] It is not surprising to note that local targeting of NAD+ signaling including enzymes such as NAMPT can enhance the efficacy of immunotherapies in several solid tumors.[43-45]

In our analysis, the identification of distinct immune profiles in PAK4-high, NAMPT-high pNENs offers clues for the development of potential targeted therapeutic approaches in the combination setting. Given the known role of mTOR inhibitors, such as everolimus, in pNEN treatment and the observed sensitivity of pNENs with aberrant PAK4/NAMPT expression to mTOR inhibition, the study implies a potential for enhanced efficacy of mTOR inhibitors in this specific subgroup. Additionally, the findings raise intriguing questions regarding the potential responsiveness of PAK4/NAMPT-aberrant pNENs to immunotherapeutic interventions. The observed immune landscape alterations in these tumors suggest a possible relationship between the PAK4/NAMPT axis and immunotherapeutic response, which warrants further investigation.

Some of the study limitations include that this a retrospective study that lacks granular clinical information. Patient outcome data have been extracted from RWE insurance claim data. Such data have been collected for purposes other than research, and thus have some limitations including increased likelihood of missing data, and variables in assessing exposures and outcomes. One limitation is that the use of deidentified data omits crucial details of the pathology (grade of differentiation and stage) that potentially could affect patient outcome. The second limitation of the study is the lack of treatment timeline that might have had a bearing effect on outcomes. The timing of tissue collection (at the time of diagnosis *v* post-treatment) and site (primary *v* metastatic) could alter the disease's biological characteristics and likely affect the PAK4 and NAMPT pathways, as metastatic sites often exhibit more aggressive traits. Unfortunately, the effect of these confounding factors on the overall survival outcomes in our cohort cannot be completely excluded. Also, in our cohort of patients, there are patients with TP53 and RB1 mutations. These mutations are rare in well-differentiated pNENs but are strongly linked to high-grade neuroendocrine carcinoma of the pancreas. Because we do not have the granular information on the grade in the commercially available tissue sets, this would confound results, given the stark biological differences between high-grade NEN and well-differentiated NENs, including survival rates and cell signaling behaviors, especially within mTOR-associated pathways. Nonetheless, the fact that we present a large data set for a rare group of malignancies using molecular analysis performed by the same NGS provider makes it relevant. Furthermore, the data presented in this report

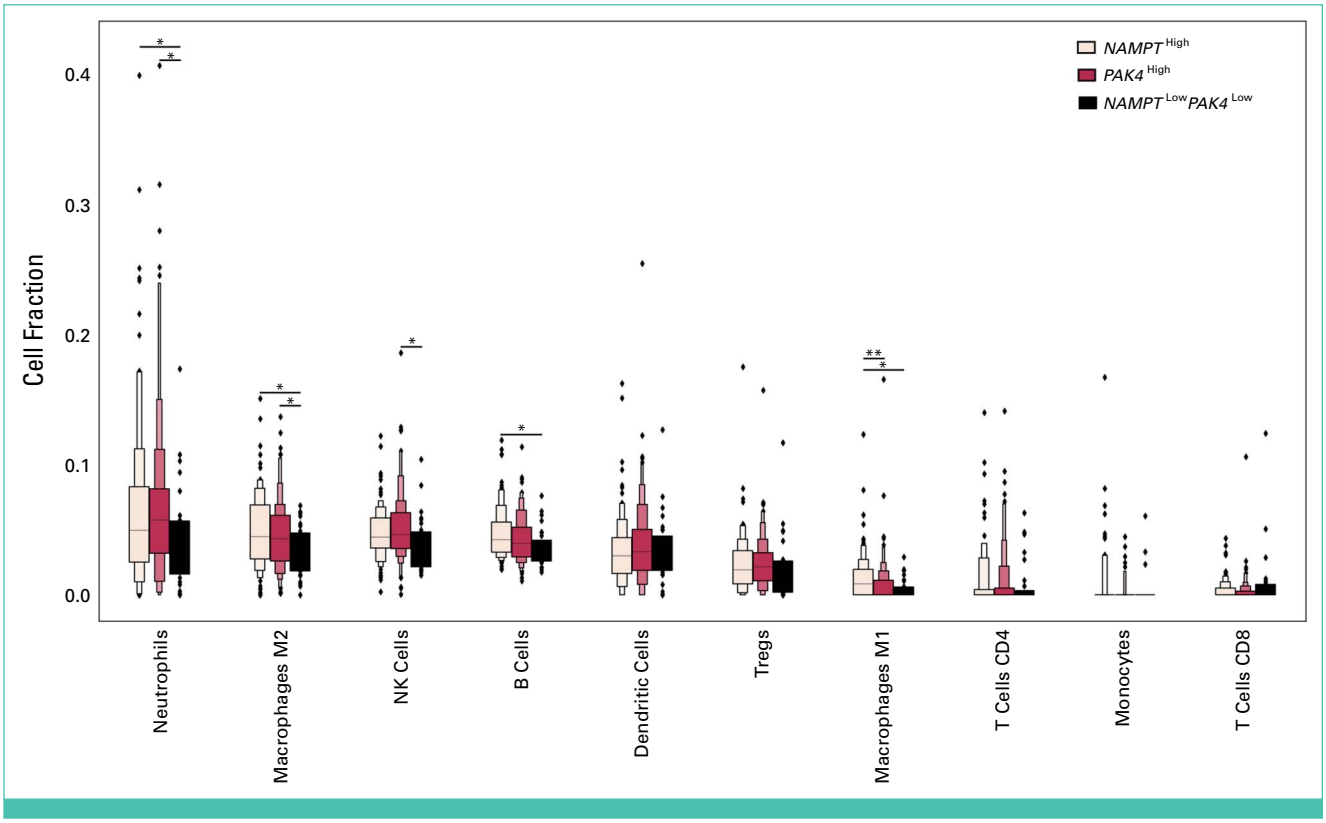

FIG 5. Box and whisker plots demonstrating the effect of PAK4 and NAMPT alterations on immune cell infiltration in pNENs. Enrichment of B cells, neutrophils, and M1 and M2 macrophages is observed in NAMPT-high and PAK4-high clusters ($q < 0.05$); however, no difference was noticed in CD4+ and CD8+ T cells across all three clusters. NAMPT, nicotinamide phosphoribosyl transferase; PAK4, p21-activated kinase 4; pNENs, pancreatic neuroendocrine neoplasms.

suggest that a sizable fraction of pNENs have high expression levels of PAK4 and NAMPT. Overall, this study potentially opens new avenues for personalized therapeutic strategies in low-grade pNENs for which there are no effective therapies, indicating the potential for tailored treatment regimens on the basis of the distinct molecular and immune profiles associated with PAK4 and NAMPT expression. PAK4-NAMPT dual inhibitor KPT-9274 has shown potent antitumor activity in preclinical models of pNENs. The drug has shown activity as a single agent or in combination with everolimus. KPT-9274 has also been shown to overcome everolimus resistance in pNEN xenograft. The findings underscore the need for further exploration and clinical trials to validate the efficacy of targeted therapies and immunotherapeutic interventions in this specific pNEN subgroup.

AFFILIATIONS

[1]IHA Hematology Oncology, Pontiac, MI
[2]Wayne State University, Detroit, MI
[3]Barbara Ann Karmanos Cancer Institute, Detroit, MI
[4]Caris Life Sciences, Phoenix, AZ
[5]University of California Irvine, Orange, CA
[6]Winship Cancer Institute of Emory University, Atlanta, GA
[7]Washington University Medical Campus, Saint Louis, MO
[8]University of Utah, Salt Lake City, UT
[9]Brown University, Providence, RI
[10]Henry Ford Health, Detroit, MI
[11]University of Alabama School of Medicine, Birmingham, AL
[12]Masonic Cancer Center/University of Minnesota School of Medicine, Minneapolis, MN
[13]Lawrence Technological University, Southfield, MI

CORRESPONDING AUTHOR

Asfar S. Azmi, PhD; e-mail: azmia@karmanos.org.

EQUAL CONTRIBUTION

I.A., H.Y.K., and S.F.B. contributed equally as first authors.

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AUTHOR CONTRIBUTIONS

Conception and design: Ibrahim Azar, Herbert Chen, Chadi Nabhan, Ramzi M. Mohammad, Asfar S. Azmi
Financial support: Alex Patrick Farrell, Ramzi M. Mohammad
Administrative support: Philip Agop Philip, Chadi Nabhan, Sanjay Goel, Ramzi M. Mohammad, Asfar S. Azmi
Provision of study materials or patients: Bassel El-Rayes, Matthew James Oberley, Anthony F. Shields, Ramzi M. Mohammad
Collection and assembly of data: Ibrahim Azar, Husain Yar Khan, Sahar F. Bannoura, Nishant Gandhi, Khalil Choucair, Nikhil Vojjala, Emil Lou, Jeffrey Swensen, Matthew James Oberley, Chadi Nabhan, Anthony F. Shields, Ramzi M. Mohammad, Asfar S. Azmi
Data analysis and interpretation: Ibrahim Azar, Sahar F. Bannoura, Nishant Gandhi, Md Hafiz Uddin, Misako Nagasaka, Jun Gong, Bassel Nazha, Khalil Choucair, Moh'd M. Khushman, Heloisa P. Soares, Wafik S. El-Deiry, Philip Agop Philip, Bassel El-Rayes, Emil Lou, Irfana Muqbil, Alex Patrick Farrell, Jeffrey Swensen, Chadi Nabhan, Sanjay Goel, Anthony F. Shields, Ramzi M. Mohammad, Boris C. Pasche, Asfar S. Azmi
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Ibrahim Azar
Honoraria: MJH Life Sciences
Consulting or Advisory Role: AstraZeneca, Genmab

Nishant Gandhi
Employment: Caris Life Sciences

Misako Nagasaka
Consulting or Advisory Role: AstraZeneca, Caris Life Sciences, Daiichi Sankyo, Takeda, Novartis, EMD Serono, Janssen, Pfizer, Lilly, Genentech, Mirati Therapeutics, Bristol Myers Squibb USA, Regeneron
Speakers' Bureau: Blueprint Medicines, Takeda, Janssen, Mirati Therapeutics
Research Funding: Tempus
Travel, Accommodations, Expenses: Anheart Therapeutics

Jun Gong
Honoraria: Astellas Pharma, Clinical Congress Consultants, QED Therapeutics, Exelixis, Elsevier, Amgen, AVEO, Basilea Pharmaceutical, EMD Serono, HalioDx, Janssen Biotech, Natera, Bayer, Pfizer/Myovant, Incyte, Eisai, Seagen, Pfizer, Agenus, Taiho Pharmaceutical, BeiGene
Consulting or Advisory Role: Amgen, Astellas Pharma, Clinical Congress Consultants, QED Therapeutics, Exelixis, Elsevier, EMD Serono/Merck, OncLive/MJH Life Sciences, Eisai, Pfizer/Myovant, Bayer, Basilea, HalioDx, Natera, Incyte, AVEO, Janssen Biotech, MJH Life Sciences, Seagen, Pfizer, BeiGene, Caper Labs, Agenus, Taiho Pharmaceutical
Patents, Royalties, Other Intellectual Property: PCT/US2021/043961 provisional patent on glutamine and cancer, US Provisional Application Serial No. 63/315,895 on methylated ctDNA markers

Bassel Nazha
Honoraria: Intellisphere, LLC (Onclive)
Consulting or Advisory Role: Exelixis, Cardinal Health, IntrinsiQ, NeoGenomics Laboratories

Moh'd M. Khushman
Stock and Other Ownership Interests: Cardiff Oncology
Consulting or Advisory Role: Muerus, TriSalus Life Sciences, Cardinal Health, Bristol Myers Squibb, AbbVie, Exelixis, Elevar Therapeutics
Speakers' Bureau: Caris Life Sciences
Research Funding: AbbVie (Inst), FOGPharma (Inst), IgM Biosciences (Inst), TORL Biotherapeutics (Inst)

Heloisa P. Soares
Honoraria: TM Isotope Technologies Munich SE
Consulting or Advisory Role: Ipsen, AstraZeneca, Novartis, Exelixis, Bristol Myers Squibb, Curium Pharma
Uncompensated Relationships: North American Neuroendocrine Tumor Society, National Comprehensive Cancer Network (NCCN), Healing NET Foundation

Wafik S. El-Deiry
Stock and Other Ownership Interests: Oncoceutics, p53-Therapeutics, Chimerix, SMURF-Therapeutics
Research Funding: Chimerix
Patents, Royalties, Other Intellectual Property: Patent on TIC10 (ONC201), Patents pending on the use of small molecules to target mutant p53, Patent on therapeutic targeting on hypoxia-inducible factors
Other Relationship: Caris Life Sciences
Uncompensated Relationships: Caris Life Sciences

Philip Agop Philip
Honoraria: Bayer, Ipsen, Incyte, Taiho Pharmaceutical, Astellas Pharma, BioNTech SE, Novocure, TriSalus Life Sciences, SERVIER, Seagen
Consulting or Advisory Role: Celgene, Ipsen, Merck, TriSalus Life Sciences, Daiichi Sankyo, SynCoreBio, Taiho Pharmaceutical
Speakers' Bureau: Incyte
Research Funding: Bayer (Inst), Incyte (Inst), Merck (Inst), Taiho Pharmaceutical (Inst), Novartis (Inst), Regeneron (Inst), Genentech (Inst), Halozyme (Inst), Lilly (Inst), Taiho Pharmaceutical (Inst), merus (Inst), BioNTech SE (Inst)
Uncompensated Relationships: Rafael Pharmaceuticals, Caris MPI

Bassel El-Rayes
Consulting or Advisory Role: Pfizer
Research Funding: Taiho Pharmaceutical (Inst), Bristol Myers Squibb (Inst), Boston Biomedical (Inst), Novartis (Inst), Hoosier Cancer Research Network (Inst), Five Prime Therapeutics (Inst), Merck (Inst), ICON Clinical Research (Inst), AstraZeneca/MedImmune (Inst), Xencor (Inst), Merck (Inst), Bayer (Inst), MedImmune (Inst), Adaptimmune (Inst), Pfizer (Inst), Novartis (Inst), IQVIA (Inst), Zymeworks (Inst), Covance (Inst), Wayne State University (Inst), Boehringer Ingelheim (Inst)

Emil Lou
Stock and Other Ownership Interests: Ryght
Honoraria: Novocure, GlaxoSmithKline, Boston Scientific, Daiichi Sankyo/UCB Japan (Inst)
Consulting or Advisory Role: Novocure, Boston Scientific
Research Funding: Novocure, Intima
Travel, Accommodations, Expenses: GlaxoSmithKline
Uncompensated Relationships: Minnetronix Medical, NomoCan, Caris Life Sciences

Alex Patrick Farrell
Employment: Caris Life Sciences
Stock and Other Ownership Interests: Caris Life Sciences

Jeffrey Swensen
Employment: Caris Life Sciences
Travel, Accommodations, Expenses: Caris Life Sciences

Matthew James Oberley
Employment: Caris Life Sciences
Leadership: Caris Life Sciences
Stock and Other Ownership Interests: Caris Life Sciences
Travel, Accommodations, Expenses: Caris Life Sciences

Chadi Nabhan
Employment: Ryght
Leadership: Ryght
Stock and Other Ownership Interests: Ryght

Sanjay Goel
Stock and Other Ownership Interests: Johnson and Johnson, Merck, Moderna Therapeutics
Honoraria: GlaxoSmithKline
Research Funding: Dragonfly Therapeutics (Inst), Deciphera (Inst), Amgen (Inst), Genentech (Inst), Xilio Therapeutics (Inst), Exelixis (Inst)
Patents, Royalties, Other Intellectual Property: I have a patent with a coinventor, John Mariadason, Ph.D, titled "Method Of Determining The Sensitivity Of Cancer Cells To EGFR Inhibitors Including Cetuximab, Panitumumab And Erlotinib.," Patent No. 20090258364

Anthony F. Shields
Consulting or Advisory Role: ImaginAb, Caris Life Sciences, Cogent Biosciences, Legend Biotech
Speakers' Bureau: Caris Life Sciences
Research Funding: Taiho Pharmaceutical, Bayer, Boehringer Ingelheim, Plexxikon, Eisai, Inovio Pharmaceuticals, H3 Biomedicine, Caris Life Sciences, ImaginAb, Exelixis, Xencor, Lexicon, Daiichi Sankyo, Halozyme, Incyte, LSK BioPharma, Esperas Pharma, Nouscom, Boston Biomedical, Astellas Pharma, AstraZeneca, Five Prime Therapeutics, MSK Pharma, Alkermes, Repertoire Immune Medicines, Telix Pharmaceuticals, Hutchison China Meditech, Seagen, Jiangsu Alphamab Biopharmaceuticals, Shanghai HaiHe Pharmaceutical, TopAlliance BioSciences Inc (Inst), Gritstone Bio (Inst), SQZ Biotechnology (Inst), Nuvation Bio (Inst), Sorrento Therapeutics (Inst), Torque (Inst), Abbisko Therapeutics (Inst), IconOVir Bio (Inst), Amal Therapeutics (Inst), TheraBionic (Inst)
Travel, Accommodations, Expenses: GE Healthcare, Caris Life Sciences, TransTarget, ImaginAb, INOVIO Pharmaceuticals

Boris C. Pasche
Employment: TheraBionic, TheraBionic (I), TheraBionic Inc (I)
Leadership: TheraBionic
Stock and Other Ownership Interests: TheraBionic

Asfar S. Azmi
Consulting or Advisory Role: Gerson Lehrman Group, Guidepoint Inc
Research Funding: Purple Biotech, FanWave, Colorado chromatography, Blackstone Therapeutics

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REFERENCES

1. SEER: Cancer Statistics. https://seer.cancer.gov/statistics/
2. Lou X, Qin Y, Xu X, et al: Spatiotemporal heterogeneity and clinical challenge of pancreatic neuroendocrine tumors. Biochim Biophys Acta Rev Cancer 1877:188782, 2022
3. Zanini S, Renzi S, Giovinazzo F, et al: mTOR pathway in gastroenteropancreatic neuroendocrine tumor (GEP-NETs). Front Endocrinol 11:562505, 2020
4. Yao JC, Shah MH, Ito T, et al: Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med 364:514-523, 2011
5. Beyens M, Vandamme T, Peeters M, et al: Resistance to targeted treatment of gastroenteropancreatic neuroendocrine tumors. Endocr Relat Cancer 26:R109-R130, 2019
6. Kumar R, Sanawar R, Li X, et al: Structure, biochemistry, and biology of PAK kinases. Gene 605:20-31, 2017
7. Radu M, Semenova G, Kosoff R, et al: PAK signalling during the development and progression of cancer. Nat Rev Cancer 14:13-25, 2014
8. Mpilla GB, Philip PA, El-Rayes B, et al: Pancreatic neuroendocrine tumors: Therapeutic challenges and research limitations. World J Gastroenterol 26:4036-4054, 2020
9. Cordover E, Wei J, Patel C, et al: KPT-9274, an inhibitor of PAK4 and NAMPT, leads to downregulation of mTORC2 in triple negative breast cancer cells. Chem Res Toxicol 33:482-491, 2020
10. Mpilla G, Aboukameel A, Muqbil I, et al: PAK4-NAMPT dual inhibition as a novel strategy for therapy resistant pancreatic neuroendocrine tumors. Cancers 11:1902, 2019
11. Yaku K, Okabe K, Hikosaka K, et al: NAD metabolism in cancer therapeutics. Front Oncol 8:622, 2018
12. Chowdhry S, Zanca C, Rajkumar U, et al: NAD metabolic dependency in cancer is shaped by gene amplification and enhancer remodelling. Nature 569:570-575, 2019
13. Winter J, Kunze R, Veit N, et al: Targeting of glucose transport and the NAD pathway in neuroendocrine tumor (NET) cells reveals new treatment options. Cancers 15:1415, 2023
14. Gasparrini M, Audrito V: Nampt: A critical driver and therapeutic target for cancer. Int J Biochem Cell Biol 145:106189, 2022
15. Alvarez MJ, Subramaniam PS, Tang LH, et al: A precision oncology approach to the pharmacological targeting of mechanistic dependencies in neuroendocrine tumors. Nat Genet 50:979-989, 2018
16. In GK, Ribeiro JR, Yin J, et al: Multi-omic profiling reveals discrepant immunogenic properties and a unique tumor microenvironment among melanoma brain metastases. NPJ Precis Oncol 7:120, 2023
17. Sammons S, Elliott A, Barroso-Sousa R, et al: Concurrent predictors of an immune responsive tumor microenvironment within tumor mutational burden-high breast cancer. Front Oncol 13: 1235902, 2023
18. Subramanian A, Tamayo P, Mootha VK, et al: Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 102: 15545-15550, 2005
19. Barbie DA, Tamayo P, Boehm JS, et al: Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. Nature 462:108-112, 2009
20. Richards S, Aziz N, Bale S, et al: Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and genomics and the Association for Molecular Pathology. Genet Med 17:405-424, 2015
21. Svejda B, Kidd M, Kazberouk A, et al: Limitations in small intestinal neuroendocrine tumor therapy by mTOR kinase inhibition reflect growth factor-mediated PI3K feedback loop activation via ERK1/2 and AKT. Cancer 117:4141-4154, 2011
22. Carracedo A, Ma L, Teruya-Feldstein J, et al: Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J Clin Invest 118:3065-3074, 2008
23. Antonuzzo L, Del Re M, Barucca V, et al: Critical focus on mechanisms of resistance and toxicity of m-TOR inhibitors in pancreatic neuroendocrine tumors. Cancer Treat Rev 57:28-35, 2017
24. Saci A, Cantley LC, Carpenter CL: Rac1 regulates the activity of mTORC1 and mTORC2 and controls cellular size. Mol Cell 42:50-61, 2011
25. Andersen JN, Sathyanarayanan S, Di Bacco A, et al: Pathway-based identification of biomarkers for targeted therapeutics: Personalized oncology with PI3K pathway inhibitors. Sci Transl Med 2: 43ra55, 2010
26. Manser E, Leung T, Salihuddin H, et al: A brain serine/threonine protein kinase activated by Cdc42 and Rac1. Nature 367:40-46, 1994
27. Yu X, Huang C, Liu J, et al: The significance of PAK4 in signaling and clinicopathology: A review. Open Life Sci 17:586-598, 2022
28. Mpilla GB, Uddin MH, Al-Hallak MN, et al: PAK4-NAMPT dual inhibition sensitizes pancreatic neuroendocrine tumors to everolimus. Mol Cancer Ther 20:1836-1845, 2021
29. Ippolito JE: Current concepts in neuroendocrine cancer metabolism. Pituitary 9:193-202, 2006
30. Duarte-Pereira S, Pereira-Castro I, Silva SS, et al: Extensive regulation of nicotinate phosphoribosyltransferase (NAPRT) expression in human tissues and tumors. Oncotarget 7:1973-1983, 2016
31. Chaker M, Minden A, Chen S, et al: Rho GTPase effectors and NAD metabolism in cancer immune suppression. Expert Opin Ther Targets 22:9-17, 2018
32. Naïja A, Merhi M, Inchakalody V, et al: The role of PAK4 in the immune system and its potential implication in cancer immunotherapy. Cell Immunol 367:104408, 2021
33. Tang L, Gao Y, Li T: Pan-cancer analysis identifies the immunological and prognostic role of PAK4. Life Sci 312:121263, 2023
34. Gajewski TF, Fessler J: PAK4 as a cancer immune-evasion target. Nat Cancer 1:18-19, 2020
35. Trott JF, Aboud OA, McLaughlin B, et al: Anti-cancer activity of PAK4/NAMPT inhibitor and programmed cell death protein-1 antibody in kidney cancer. Kidney360 1:376-388, 2020
36. Abril-Rodriguez G, Torrejon DY, Liu W, et al: PAK4 inhibition improves PD-1 blockade immunotherapy. Nat Cancer 1:46-58, 2020
37. Abril-Rodríguez G, Torrejon DY, Karin D, et al: Remodeling of the tumor microenvironment through PAK4 inhibition sensitizes tumors to immune checkpoint blockade. Cancer Res Commun 2: 1214-1228, 2022
38. Ma W, Wang Y, Zhang R, et al: Targeting PAK4 to reprogram the vascular microenvironment and improve CAR-T immunotherapy for glioblastoma. Nat Cancer 2:83-97, 2021
39. Navas LE, Carnero A: NAD^+ metabolism, stemness, the immune response, and cancer. Signal Transduct Target Ther 6:2, 2021
40. Lv H, Lv G, Chen C, et al: NAD^+ metabolism maintains inducible PD-L1 expression to drive tumor immune evasion. Cell Metab 33:110-127.e5, 2021

41. Audrito V, Serra S, Brusa D, et al: Extracellular nicotinamide phosphoribosyltransferase (NAMPT) promotes M2 macrophage polarization in chronic lymphocytic leukemia. Blood 125:111-123, 2015
42. Pylaeva E, Harati MD, Spyra I, et al: NAMPT signaling is critical for the proangiogenic activity of tumor-associated neutrophils. Int J Cancer 144:136-149, 2019
43. Li M, Kirtane AR, Kiyokawa J, et al: Local targeting of NAD^+ salvage pathway alters the immune tumor microenvironment and enhances checkpoint immunotherapy in glioblastoma. Cancer Res 80:5024-5034, 2020
44. Wu Y, Pu C, Fu Y, et al: NAMPT-targeting PROTAC promotes antitumor immunity *via* suppressing myeloid-derived suppressor cell expansion. Acta Pharm Sin B 12:2859-2868, 2022
45. Travelli C, Colombo G, Aliotta M, et al: Extracellular nicotinamide phosphoribosyltransferase (eNAMPT) neutralization counteracts T cell immune evasion in breast cancer. J Immunother Cancer 11:e007010, 2023

APPENDIX

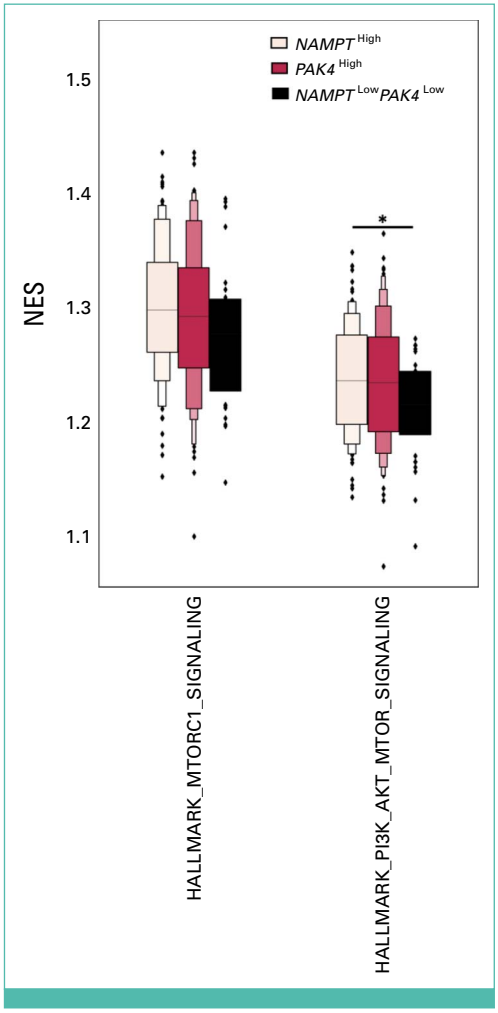

FIG A1. Box and whisker plots representing single-set enrichment analysis representing changes in hallmark (MTORC1) and hallmark PI3K mTOR signaling in NAMPT-high, PAK4-high versus NAMPT-low and PAK4-low groups. It was found that there is no statistically significant difference between the groups. mTOR, mammalian target of rapamycin; NAMPT, nicotinamide phosphoribosyl transferase; NES, normalized enrichment score; PAK4, p21-activated kinase 4.

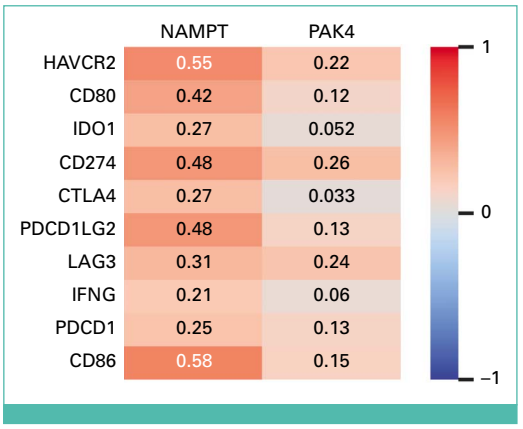

FIG A2. Heat map showing the expression in the association of PAK4 and NAMPT expression with immune checkpoint gene expression and immune cell infiltration. A moderate positive correlation was observed between the expressions of HAVCR2 and CD86 and NAMPT expression change in immune genes in NAMPT-high and PAK4-high groups in contrast to LAG3, CD80, and IDO1 as represented in Figure 4. NAMPT, nicotinamide phosphoribosyl transferase; PAK4, p21-activated kinase 4.

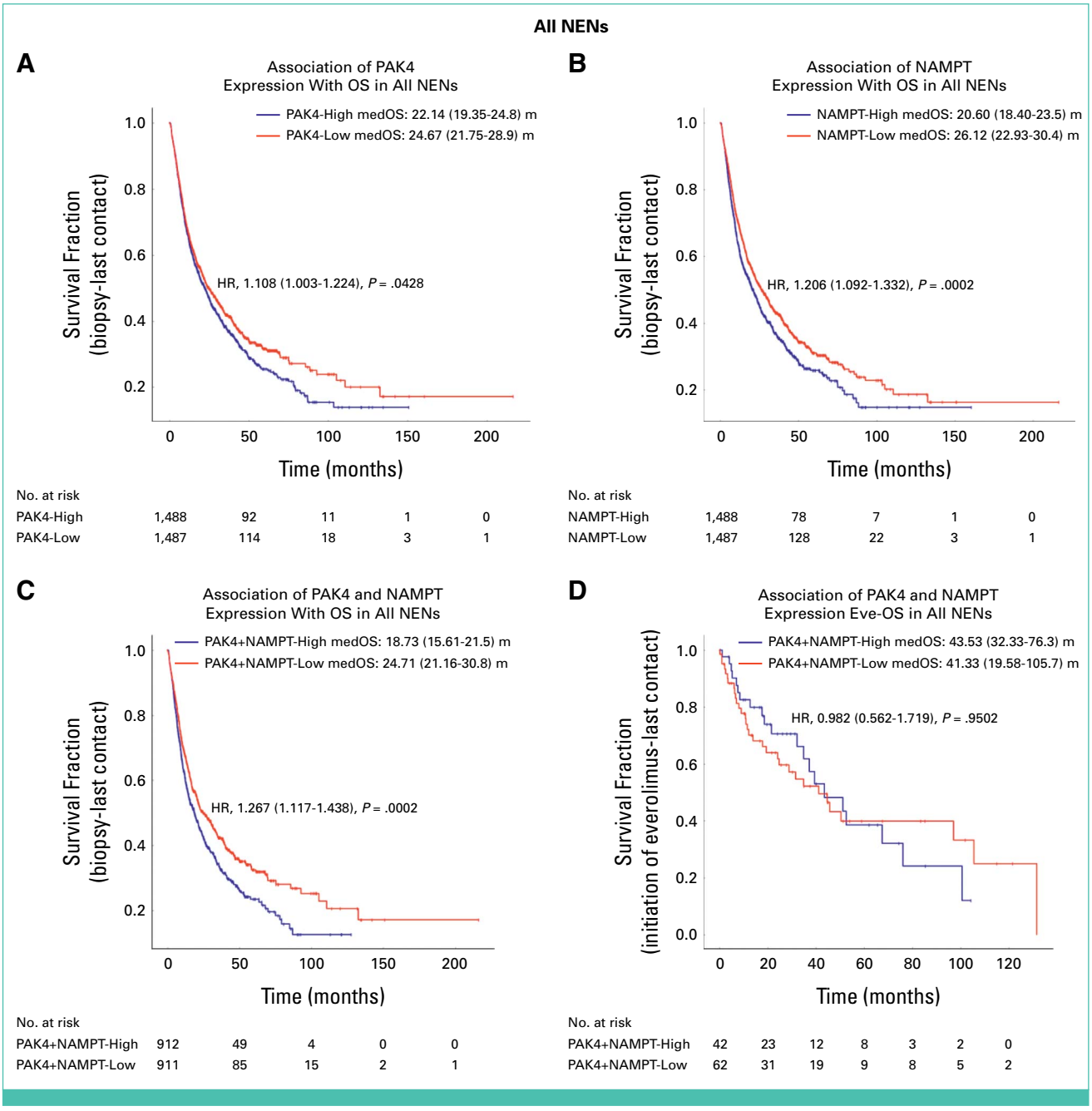

FIG A3. Kaplan-Meier survival analysis of PAK4 and NAMPT expression in NENs. (A-D) In patients with NEN, either high PAK4 or NAMPT is associated with lower overall survival compared with their low-expression cohorts ($P < .05$). However, in pNENs, similar trends are observed but not statistically significant ($P > .05$; E-H). HR, hazard ratio; NAMPT, nicotinamide phosphoribosyl transferase; NENs, neuroendocrine neoplasms; OS, overall survival; PAK4, p21-activated kinase 4; pNENs, pancreatic neuroendocrine neoplasms. (continued on following page)

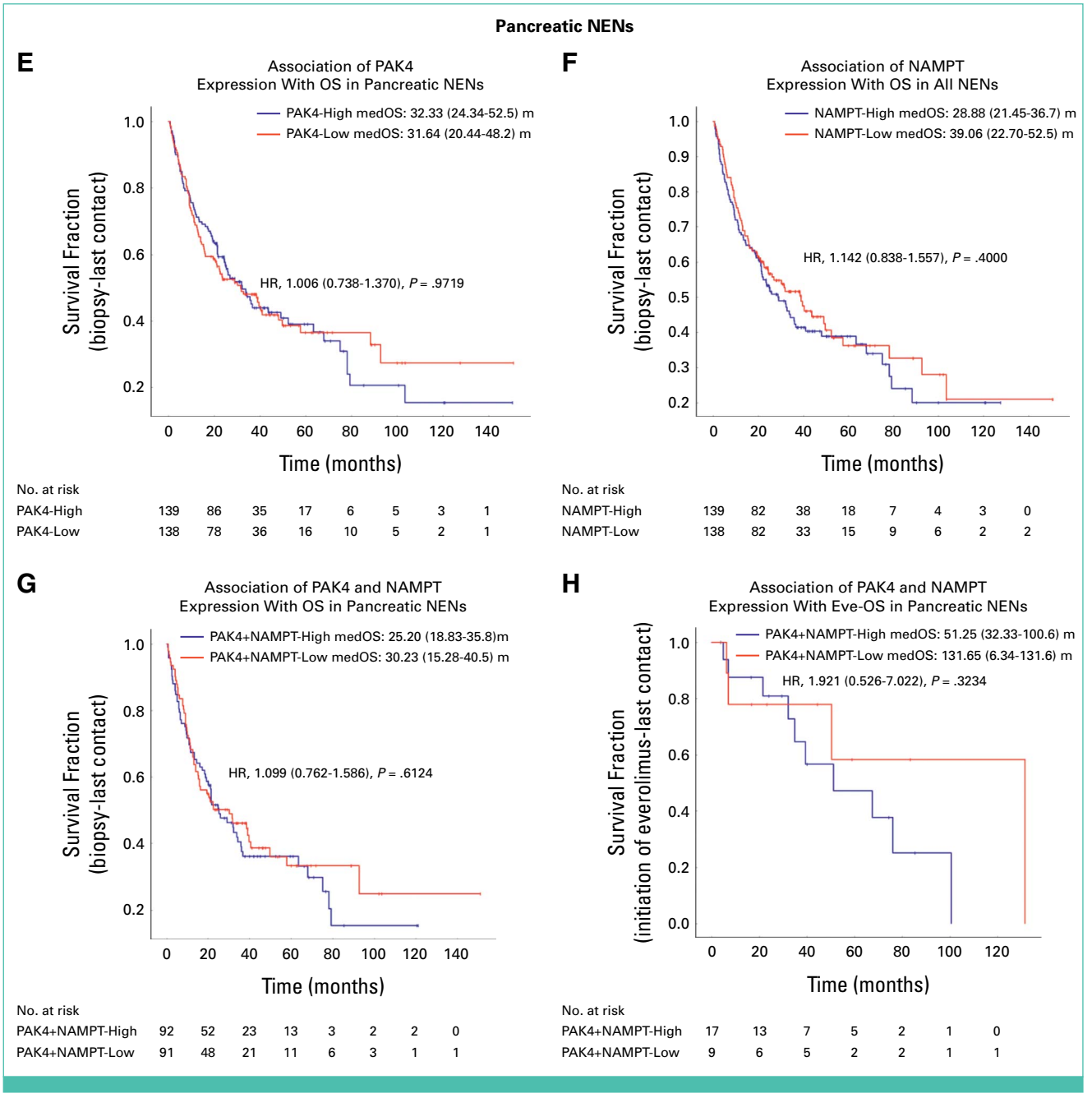

FIG A3. (Continued).

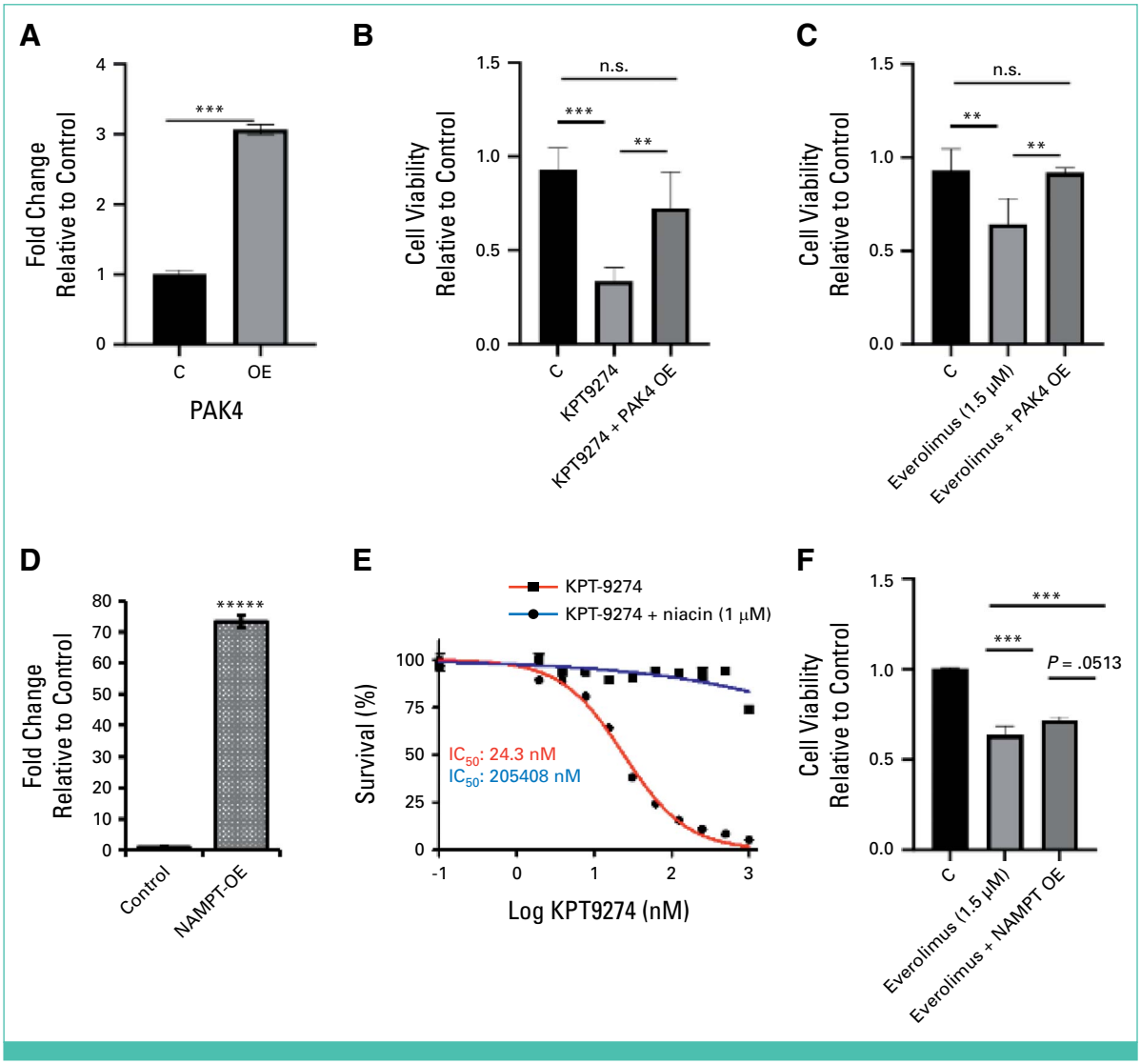

FIG A4. (A) RT-PCR showing OE of PAK4 in BON-1 cells after plasmid introduction. (B) MTT assay at 72 hours showing statistically significant reduced response to KPT-9274 in PAK4 OE cells. (C) MTT assay at 72 hours showing statistically significant low response to everolimus. (D) RT-PCR showing OE of NAMPT after plasmid introduction. (E) MTT assay at 72 hours showing lack of growth inhibition when KPT9274 was coadministered with niacin. (F) MTT assay at 72 hours showing diminished activity of everolimus in NAMPT overexpressing cells. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NAMPT, nicotinamide phosphoribosyl transferase; OE, overexpression; PAK4, p21-activated kinase 4; RT-PCR, reverse transcriptase polymerase chain reaction. ***P* < .05, ****P* < .01, *****P* < .0001.

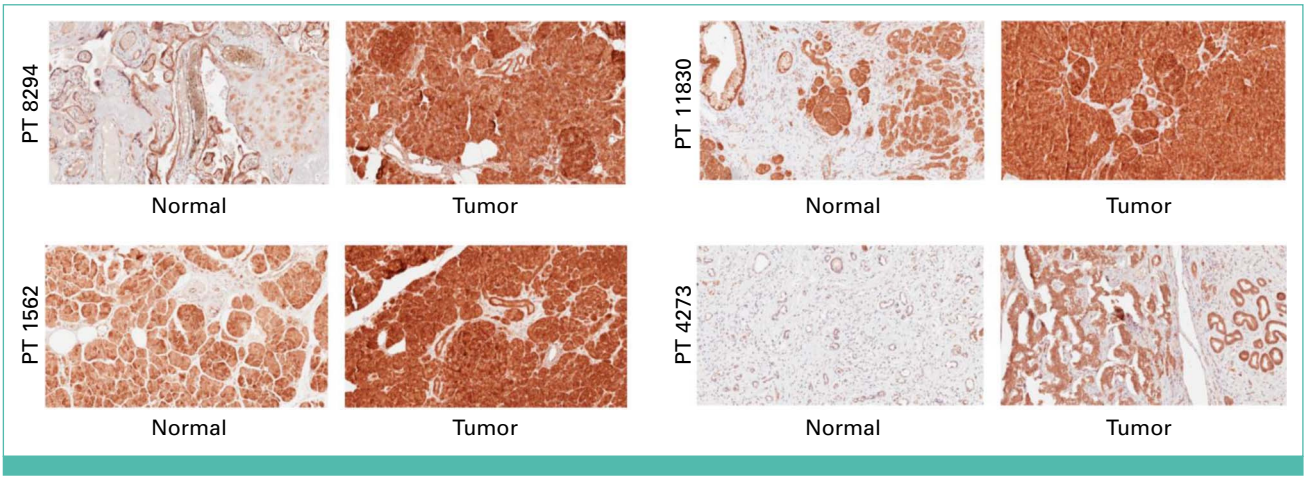

FIG A5. Immunohistochemistry staining preparations showing expression of PAK4 in pNEN tumors. It represents a higher intensity of staining of PAK4 in pNEN compared with matched control. PAK4, p21-activated kinase 4; pNENs, pancreatic neuroendocrine neoplasms.