Nicotinamide Phosphoribosyl Transferase and Cancer: A Molecular Nutrition Link

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Abstract: Nicotinamide phosphoribosyl transferase (NAMPT, EC 2.4.2.12), a pleiotropic protein, is a key enzyme for cellular NAD synthesis and may function as a growth factor or cytokine. Dysregulation of NAMPT gene expression has been implicated in several human diseases. In recent years, an increasing attention has been drawn to NAMPT’s role in the pathogenesis of cancer and its value as a therapeutic target in cancer therapy. A molecular nutrition link between NAMPT and cancer looms large among the scientific findings. This review article will be centered on this theme. We will summarize the genomic discoveries of NAMPT in cancer and the current understanding of molecular mechanisms underlying NAMPT in the pathogenesis of cancer, especially on a molecular nutrition link. We will also synopsize several small molecule inhibitors of NAMPT and their potential therapeutic utilities in cancer and usher in some terra incognita of future researches on NAMPT in cancer.

Key Words: NAMPT; Cancer; PBEF; Visfatin; Growth factor; Cytokine

Introduction

Nicotinamide phosphoribosyl transferase (NAMPT, EC 2.4.2.12) is a pleiotropic protein [1]. It is an enzyme that catalyzes the condensation of nicotinamide with 5-phosphoribosyl 1-pyrophosphate (PRPP) to yield nicotinamide mononucleotide (NMN), a rate-limiting enzyme in a mammalian salvage pathway of nicotinamide adenine dinucleotide (NAD) synthesis [2]. NAMPT may also function as a growth factor or cytokine [3]. NAMPT is initially named pre-B-cell colony-enhancing factor (PBEF) for its growth-factor-like function on promoting pre-B-cell colony formation in the presence of stem cell factor plus interleukin 7 [3]. Later, NAMPT is characterized as a novel cytokine [4], which stimulates the expression of IL-6, IL-8, and other inflammatory cytokines [4, 5]. In 2005, NAMPT/PBEF was given another tantalizing name visfatin, a “new visceral fat-derived hormone”, which is an adipocyte-derived adipokine that induces insulin mimetic effects [6]. Although its insulin mimetic function is disputed, visfatin has engendered a unflagging enthusiasm for its physiological role in cell metabolism and its association with obesity and diabetes [1]. Interested readers, who desire more in-depth knowledge on the discovery, physiological functions, and pathological roles of NAMPT, may refer to our earlier comprehensive review article [1]. To avoid any confusion, the name NAMPT will be used throughout this review article since NAMPT has been approved as the official name of this gene by the Human Genome Organization Gene Nomenclature Committee (HGNC: 30092, www.genenames.org).

In recent years, an increasing attention has been drawn to NAMPT’s role in the pathogenesis of cancer and its utility as a therapeutic target in cancer therapy [7]. When searching for “NAMPT or PBEF or Visfatin AND Cancer” in PubMed (www.ncbi.nlm.nih.gov/PubMed), there were 326 articles published on the subject as of Jan. 25, 2017. As of Jan. 25, 2017, there had been 70 published records on ‘NAMPT or PBEF or Visfatin AND Polymorphisms’ in the PubMed. Among the scientific findings, a molecular nutrition link between NAMPT and cancer looms large. This review article will be centered on this theme. The first part will briefly cover the genomic discoveries of NAMPT in cancer. The bulk of the data are derived from high-throughput omics analyses. The second part will be devoted to the current understanding of molecular mechanisms underlying NAMPT’s roles in the pathogenesis of cancer. A molecular nutrition link between NAMPT and cancer will be the linchpin. The third part will synopsize several small molecule inhibitors of NAMPT and their therapeutic utilities in cancer therapy. The focus will be the strategic applications of NAMPT inhibitors in cancer therapy. The final part will usher in some Terra Incognita of future investigations on NAMPT in Cancer.

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Genomic Discoveries of NAMPT in Cancer

In this section, we will briefly introduce normal NAMPT gene structure, NAMPT gene expression in normal and cancer tissues, and NAMPT Gene Coding Mutations in Cancers. Most data are derived from genomic discoveries.

Normal NAMPT Gene Structure

The human NAMPT gene maps cytogenetically to 7q22.3. It spans 38.04 kb on the complement strand of Chromosome 7: 106,248,285-106,286,326 (CRCh38.p7). According to the Ensemble gene annotation system, there are 13 NAMPT (ENSG00000105835) transcripts or isoforms, consisting of 4 protein coding, 5 processed, and 4 retained intron transcripts [8]. The primary transcript (NAMPT-001; ENST00000222553.7), which contains 11 exons and 10 introns, is 4582-bp long and encodes a 491-amino acid, 55.5-Kd protein (NP_005737.1, EC 2.4.2.12) (Figure 1A). The functions of the remaining 12 secondary transcripts, including the 3 transcripts which encode predicted proteins of 368, 88, and 60 amino acids, respectively, remain to be elucidated.

Ognjanovic is the first to characterize the human NAMPT promoter region [9]. One major and several minor transcriptional start sites (TSS) are identified by primer extension. Sequence analysis of the 5' upstream region reveals two distinct regulatory segments: a 1.4-kb, GC rich (60%) proximal region and 1.6-kb, GC poor (40%) distal region.

Figure 1. NAMPT gene structure, expression, and mutations in cancer. A. Genomic organization of the 11 exons and 10 introns of the human NAMPT gene. The number of amino acids encoded by each exon are listed above the exon, while the length (bp) of each intron is listed below. The gene is presented left to right; however, the orientation of the gene is on the reverse or complementary strand of Chromosome 7. B. Expression of NAMPT in cancer tissues. The frequencies of NAMPT over-expression and under-expression in cancerous tissues relative to normal tissues were plotted using BioXpress v1.0. Abbreviations: ESCA (Esophageal carcinoma), CESC (Cervical squamous cell carcinoma and endocervical adenocarcinoma), UCEC (Uterine Corpus Endometrial Carcinoma), LIHC (Liver hepatocellular carcinoma), KIRP (Kidney renal papillary cell carcinoma), KIRC (Kidney renal clear cell carcinoma), KICH (Kidney Chromophobe), PRAD (Prostate adenocarcinoma), READ (Rectum adenocarcinoma), PAAD (Pancreatic adenocarcinoma), THCA (Thyroid carcinoma), BRCA (Breast invasive carcinoma), LUSC (Lung squamous cell carcinoma), LUAD (Lung adenocarcinoma), HNSC (Head and Neck squamous cell carcinoma), BLCA (Bladder Urothelial Carcinoma), STAD (Stomach adenocarcinoma), PRAD (Prostate adenocarcinoma). C. Somatic mutations and copy-number alterations of human NAMPT gene in cancer. Frequency of copy-number and somatic alterations in NAMPT in different cancer types as queried in the eBioPortal. The top 25 cancer types containing an alteration frequency greater than 2.4% are depicted. Study name abbreviations: BCCRC (Breast cancer patient xenografts; British Columbia), NEPC (Neuroendocrine Prostate Cancer, Trento/Cornell/Broad), ESCA (Esophageal Carcinoma, TCGA, Provisional), NCI-60 (NCI 60 cancer cell lines), CCLE (Cancer Cell Line Encyclopedia, Novartis/Broad), STAD-1 (Stomach Adenocarcinoma, TCGA, Provisional), PRAD-1 (Prostate Adenocarcinoma, Metastatic, Michigan), STAD-2 (Stomach Adenocarcinoma, TCGA), HNSC-1 (Head and Neck Squamous Cell Carcinoma, TCGA), LUSC-1 (Lung Squamous Cell Carcinoma, TCGA, Provisional), OV (Ovarian Serous Cystadenocarcinoma, TCGA, Provisional), SKCM (Skin Cutaneous Melanoma, TCGA, Provisional), LUAD-2 (Lung Adenocarcinoma, TCGA, Provisional), chRCC (Kidney Chromophobe, TCGA, Provisional), HNSC-2 (Head and Neck Squamous Cell Carcinoma, TCGA, Provisional), PRAD-2 (Prostate Adenocarcinoma, Fred Hutchinson CRC), UCEC (Uterine Corpus Endometrial Carcinoma, TCGA, Provisional), CHOL (Cholangiocarcinoma, TCGA, Provisional), BLGG (Brain Lower Grade Glioma, TCGA, Provisional), PAAD (Pancreatic Cancer, UTSW), NSCLC (Panc-Lung Cancer, TCGA), SARC-2 (MSKCC/Broad). Interested readers can go to The cBioPortal for Cancer Genomics site (www.cbioportal.org) to get details of these studies, which provides visualization, analysis and download of large-scale cancer genomics data sets.
elements [9]. The ENCODE (Encyclopedia of DNA Elements) Consortium has characterized further the NAMPT regulatory regions [10]. The presence of modified histone sites, DNase I accessibility sites, and transcription factor binding sites have been detected throughout the NAMPT gene. Acetylated histone (H3K27Ac) binding, an indicator of active expression, is strongly evident around exon 1. There are 2608 annotated genetic variants, including 2213 single nucleotide polymorphisms (SNPs), in the 44.94-kb region of Chromosome 7: 106,244,540-106,289,477, which contains the flanking upstream and downstream regulatory regions of the NAMPT gene (http://www.ncbi.nlm.nih.gov/variation/view/?q=10135[geneid, CRCh38.p7]). Of the 1270 variants, whose frequency has been determined, 164 have a minor allele frequency (MAF) > 1%. Our group has contributed the first report in the literature that identifies 11 SNPs in the human NAMPT gene proximal promoter by direct DNA sequencing from 36 people [11]. We have then genotyped two SNPs in a Caucasian population and found that carriers of the haplotype GC from SNPs T-1001G and C-1535T (C-1535T was mislabeled as C1543T in the original publication) in the human NAMPT gene promoter have a 7.7-fold higher risk of acute respiratory distress syndrome (ARDS), while the haplotype TT represents a protective haplotype to ARDS [11].

As of Jan. 25, 2017, there had been 70 published records on ‘NAMPT or PBEF or Visfatin AND Polymorphisms’ in the PubMed. Most of these reports are on the association of NAMPT SNPs with obesity, diabetes, and cardiovascular diseases, except one study in which haplotypes CTC, CTT, and CAC of SNPs (rs61330082, rs2505568, and rs9034) of the NAMPT gene were correlated significantly with esophageal squamous cell carcinoma susceptibility [12]. To date, only 165 of 2608 annotated variants derived from genomic studies have been addressed in the literature, demonstrating that there is still more investigations needed to understand the physiological consequence of the variants, especially in the areas of cancer research.

NAMPT Gene Expression in Normal Tissues and Cancer

NAMPT is expressed in multiple tissue types. A compilation of 13 expression studies, available through the Expression Atlas (www.ebi.ac.uk), has demonstrated that NAMPT is expressed in 133 different human tissues [13]. The Expression Atlas normalizes the expression levels across all studies, which allows for reliable meta-analysis [13]. Across all the 13 studies, NAMPT is most highly expressed in bone marrow, whole blood, appendix, and lungs [13]. Review of the Illumina Body Map 2.0, a dataset of RNA-Seq of human individual tissues and mixture of 16 tissues, has revealed that NAMPT expression is highest in lungs, leukocytes, adipose, and liver tissues (www.ebi.ac.uk/gxa/experiments/E-MTAB-513). Differential NAMPT expression relative to normal tissue has been detected in multiple diseases, as reviewed in Zhang LQ [1].

The overexpression of NAMPT gene has been well documented in a number of tumors, including colorectal [14], ovarian [15], and prostate cancer [16], astrocytomas [17] and melanoma [18]. Overexpression of NAMPT has also been noted to correlate with more aggressive lymphomas [19] and high-grade astrocytomas [17], and with poor outcome in gastric [20] and endometrial carcinomas [21]. In addition, the overexpression of NAMPT gene has been linked to bortezomib-resistance in multiple myeloma cells [22] and etoposide-resistance in vitro [23]. Tumor cell sensitivity to NAMPT inhibition is inversely proportional to the level of NAMPT expression [19, 24-26]. High-throughput omics approaches have curated more types of cancers with NAMPT gene overexpression though it is variable [27]. Comparisons of the frequencies of cancers with overexpression or underexpression of NAMPT relative to normal tissues validate the heterogeneous nature of cancer (Figure 1B), which is derived from a curated database [28], BioXpress v1.0. Of the 18 cancer types in which curated RNA-seq data are available, nine have an overexpression frequency greater than 50%, while the remaining nine have an underexpression frequency greater than 50%. These results indicate the complexity of NAMPT gene regulation in the context of both tissue and cancer biotypes. The genetic underpinning of the upregulation of NAMPT gene in cancers is largely unknown. There is a paucity of population and molecular studies on whether there are some single nucleotide polymorphisms (SNPs) in the NAMPT gene promoter, intron or 3′-untranslated regions in cancer patients, which may be the culprits responsible for the NAMPT gene dysregulation in cancers. The relationship between copy number aberration (CNA) and NAMPT gene expression levels in cancer is enigmatic [27]. For example, the amplification of NAMPT in neuroendocrine prostate cancer does not result in an increase in NAMPT mRNA expression, as measured by RNA-Seq, compared to slight gain of copy numbers and normal diploid copy numbers [27]. However, in esophageal carcinoma, NAMPT mRNA expression is elevated in tumor samples with increased CNAs (gain, amplification) relative to diploid cancer genotypes [27]. Shallow deletions, also known as loss of heterozygosity, result in lower expression of NAMPT in esophageal carcinoma [27]. These results indicate the complexity of NAMPT gene regulation in the context of both tissue and cancer biotypes.

NAMPT Gene Coding Mutations in Cancers

Gene alterations are common findings in cancer. To determine the frequency of alterations within the NAMPT gene, we have performed a cross-cancer query using the cBioPortal for Cancer Genomics (http://cbioportal.org) for both somatic mutations and DNA copy-number alterations (CNAs) [27] (Figure 1C). A query of 141 cancer datasets has identified 77 somatic mutations, consisting of 62 missense, 7 nonsense, 4 frame shift deletions, and 4 splice
sight alterations [27]. The cancer types that most frequently contain somatic mutations are cholangiocarcinoma (2.9%), diffuse large B-cell lymphoma (1.7%), and lung squamous cell carcinoma (1.7%). There are 7 missense mutations predicted to be highly damaging [27]. The distribution of the somatic mutations along the NAMPT protein does not reveal a major mutational hotspot, although the majority of alterations exist in the conserved enzymatic domain [27]. A query for CNAs has revealed a high frequency of NAMPT gene amplification in neuroendocrine prostate cancer (15.9%), esophageal carcinoma (9.2%), and ovarian serous cystadenocarcinoma (5.9%). The NAMPT amplifications are also a frequent finding in the NCI-60 cell lines (8.3%) [27]. Deletions are also detected, often times in cancer types that also present with amplifications, such as ovarian serous cystadenocarcinoma (0.2%) and stomach adenocarcinoma (0.3%) (Figure 1C) [29]. Besides a few coding mutations, such as those in residues 93, 165,191, 217, and 388 of the NAMPT protein, which are associated with cancer resistance to NAMPT inhibitors by either affecting NAMPT’s substrate binding or allosteric interaction [24, 26, 30, 31], the mechanisms by which most of these NAMPT somatic mutations and CNAs via genomic discoveries are involved in cancer biology remain to be investigated and validated.

Molecular Nutrition Link between NAMPT and Cancer
Clinical surveys, experimental investigations and pharmacological studies have provided solid evidence to link NAMPT with tumor pathogenesis, progression, and outcomes, suggesting that it be a therapeutic target [32]. Although underlying molecular mechanisms of these linkages are far from being fully appreciated, accumulated findings have ascribed at least three pathological roles of NAMPT in cancer: anti-apoptosis, pro-angiogenesis, and pro-inflammatory [1]. These roles are best explained by NAMPT’s enzymatic activity to augment cellular NAD synthesis and hence cellular metabolisms, as well as NAMPT’s non-enzymatic functions, such as inflammatory cytokine activity in obesity-related cancer patients. Thus the discussion in the following section will be focused on a molecular nutritional link between NAMPT and cancer.

Link between NAMPT and NAD Link
As aforementioned, NAMPT is an essential gene. Knockout of the Nampt gene in mice results in embryonic lethality [6]. Further, when the Nampt gene-floxed adult mice with Tamoxifen-induced Cre are subjected to the Tamoxifen treatment, their Nampt genes are homozygously knocked out and the mice die within 10 days (Ye Unpublished data). These evidences corroborate the Nampt gene’s essentiality for the life. NAMPT’s essential role perhaps lies on that it catalyzes a rate-limiting step in a mammalian salvage pathway of nicotinamide adenine dinucleotide (NAD) synthesis [33]. Upregulation of NAMPT gene expression and hence enhanced synthesis of NAD have been a frequent findings in cancer patients [32]. The NAD metabolome is a key determinant in cancer cell biology [34]. This is because the enhanced synthesis of NAD⁺/NADH in tumor cells is a key adaptation to meet their uncontrolled proliferation needs of energy as NAD⁺/NADH acts as a coenzyme to be rapidly oxidized and reduced to generate cellular energy currency, i.e., ATP. Furthermore, it is increasingly recognized that NAD also serves as the substrate for important cancer-related enzymes such as ADP-ribose transferases, including poly-ADP-ribose polymerases (PARPs), sirtuins, and cyclic ADP (cADP) ribose synthases [33]. These enzymes are important in DNA repair, G-protein coupled receptor signaling, calcium homeostasis, transcriptional regulation, and ultimately cancer cell survival [35].

One of the hallmarks of human cancers is the intrinsic or acquired resistance to apoptosis [36]. Evasion of apoptosis may contribute to carcinogenesis, tumor progression, and treatment resistance [37]. NAMPT inhibits apoptosis of tumor cells via its role as a key enzyme in NAD biosynthetic salvage pathway [33]. Administration of NAD has been demonstrated to protect the cells from apoptosis in differentiated PC12 cells [38], neutrophils [39], cochlear axons and sensory hair cells [40], HeLa, RAW, and HepG2 cells [41], neuronal cells [42], while depletion of NAD causes apoptosis in B16-BL6 melanoma cells [43], THP-1 cells [44], mammary carcinoma [45], and other cells [46]. NAD may affect apoptosis through several potential mechanisms [47]. First, NAD mediates cellular energy metabolism that is a critical factor determining cell death modes. Second, the NADH/NAD⁺ ratio is a major index of cellular reducing power that affects mitochondrial permeability transition(MPT), a mediator of apoptosis under many conditions. Third, NAD⁺ levels mediate the activity of caspase-dependent endonuclease DNA fragmentation factor 40 (DFF40), an executioner of DNA fragmentation in certain apoptotic cascades. Finally, NAD⁺-dependent sirtuins may mediate apoptosis [48]. Future studies on this topic are critical for our comprehensive understanding about the roles of NAMPT in anti-apoptosis in tumor cells via the NAD synthesis.

The growth of cancers is dependent upon the formation of new blood vessels, a process including angiogenesis. Angiogenesis is a dominant process supporting tumor growth. Neovascularization by angiogenesis is the prerequisite for the expansion of tumor [49]. Kim SR [50] has found that NAMPT potently stimulates in vivo neovascularization in chick chorioallantoic membrane and mouse Matrigel plug. They have also demonstrated that NAMPT activates migration, invasion, and tube formation in human umbilical vein endothelial cells (HUVECs). Moreover, NAMPT evokes activation of the ERK1/2 in endothelial cells, which is closely linked to angiogenesis [51]. Inhibition of ERK activation markedly decreases NAMPT-induced tube formation of HUVECs and NAMPT-stimulated endothelial cell sprouting from rat aortic rings [51]. Taken together, these
Our previous work has provided evidence that regulation of lung injury [11], sepsis [71], and rheumatoid arthritis [72]. In several common inflammatory diseases, such as acute inflammation of NAMPT gene expression has been well documented. Upregulation of inflammatory cytokines, including IL-1β [66], IL-6 [67, 68], TNF-α, and IL-8 [68], are associated with increased breast cancer tumor growth and poor patient outcomes. The same cytokines have all been shown to be upregulated in obese white adipose tissue [69, 70]. As stated above, the NAMPT expression is upregulated in both obesity and cancer. NAMPT may play a key bridge role between obesity and cancer via its inflammatory cytokine activity. NAMPT has been characterized as a novel cytokine [4], which stimulates the expression of IL-6, IL-8, and other inflammatory cytokines [4, 5]. Upregulation of NAMPT gene expression has been well documented in several common inflammatory diseases, such as acute lung injury [11], sepsis [71], and rheumatoid arthritis [72]. Our previous work has provided evidence that regulation of inflammatory cytokine expression in pulmonary epithelial cells by NAMPT is via a nonenzymatic and AP-1-dependent mechanism [5]. Soncini D [73] has also reported that NAMPT promotes epithelial-to-mesenchymal transition, independent of its enzymatic activity. Other studies [74, 75] have also corroborated NAMPT’s non-enzymatic functional activities. A recent review by Grolla AA [32] has an excellent rendition of extracellular NAMPT as a new cancer metabolite, which has updated the current understanding of extracellular NAMPT’s immunometabolic function, secretion, mechanism of action, and possible roles in the cancer micro-environment. Further studies are warranted to gain a deeper glean into the detailed and comprehensive molecular mechanisms underlying the bridging role of NAMPT between obesity and cancer.

**NAMPT Inhibitors and Their Therapeutic Utilities in Cancer**

As summarized above, the overexpression of NAMPT has been involved in tumor pathogenesis, progression, and outcome. Thus, NAMPT has become an attractive target in the treatment of cancer. This section will focus on the small molecule inhibitors to NAMPT.

**FK866 as the First Small Chemical Inhibitor of NAMPT**

The first NAMPT inhibitor, FK866 (also called APO866 or WK175, (E)-N-[4-(1-benzoylpyrerpiperidin-4-yl) butyl]-3-(pyridin-3-yl)], was reported by Hasmann and Schemainda, who found that FK866 induced apoptosis by a highly specific and potent inhibition of nicotinamide phosphoribosyltransferase to deplete the cells of a vital factor, NAD, in human liver carcinoma HepG2 cells [76]. Depletion of cellular NAD levels leads to a lowered ATP level and the inhibition of poly (ADP-ribose) polymerases (PARPs) [77, 78]. Cancer cells have a high demand of both PARP and ATP, and display high energy requirements [78]. Thus, cancer cells would be expected to be more sensitive than normal cells to the inhibition of NAD synthesis. FK866 has been shown to have anti-tumor, anti-metastatic and anti-angiogenic activities in a murine renal cell carcinoma model [79]. Because FK866, which inhibits the enzymatic activity of NAMPT displays strong anticancer activity both in vitro and in vivo, it has stimulated the investigative expansion of discovering more small chemical inhibitors to NAMPT. Table 1 presents a partial list of newly discovered small molecule inhibitors of NAMPT. Interested readers may trace back to the original publications for the details.

In the initial clinical trials, no objective positive responses were seen with FK866 or CHS-828 to treat advanced solid tumors or with GMX1777 to treat advanced malignancies, although some patients exhibited stable disease [80-82]. There are the most frequently observed toxicities with these drug trials including thrombocytopenia and gastrointestinal toxicities such as diarrhea, vomiting, and esophagitis [83]. One possible reason for the lack of efficacy is that insufficient doses of NAMPT inhibitors could be achieved for...
clinical efficacy when administered as a single agent. Another possibility is that the dosing schedule is not optimized to attain the threshold duration and degree of NAD\(^+\) depletion required to cause cytotoxicity in tumor cells [35].

**Table 1. Exemplary small molecule inhibitors of NAMPT**

<table>
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<tr>
<th>PMID *</th>
<th>Inhibitor Name</th>
<th>IC50 (nM)</th>
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<tr>
<td>21080724</td>
<td>CB-30865</td>
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</tr>
<tr>
<td>21884623</td>
<td>3-amino-2-benzyl-7-nitro-4-(2-quinoyl)-1,2-dihydroisouquinolin-1-one</td>
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<tr>
<td>21330015</td>
<td>FK866 analogs-compound 7</td>
<td>0.3-4.86</td>
</tr>
<tr>
<td>22889195</td>
<td>Three carbazole based agents</td>
<td>0.31-2.20</td>
</tr>
<tr>
<td>24164086</td>
<td>New analogues compounds</td>
<td>0.025-0.33</td>
</tr>
<tr>
<td>23896914</td>
<td>2,3-dihydro-1H-pyrrlo[4,3-c]pyridine-derived ureas (18, 29)**</td>
<td>7-35</td>
</tr>
<tr>
<td>23668988</td>
<td>Urea-containing NAMPT inhibitors (17)</td>
<td>3-70</td>
</tr>
<tr>
<td>24183972</td>
<td>STF-118804</td>
<td>2.6-62</td>
</tr>
<tr>
<td>24021463</td>
<td>Amides derived from 1H-pyrrozol[3,4-b]pyridine-5-carboxylic acid(GNE-618, 26)**</td>
<td>4.3-6.1</td>
</tr>
<tr>
<td>23859118</td>
<td>Nampt-7 (58)</td>
<td>9-10</td>
</tr>
<tr>
<td>23617784</td>
<td>Athiourea analogue(50)</td>
<td>7-3.2</td>
</tr>
<tr>
<td>24900822</td>
<td>Thiazolocarboxamide Analogues (5,27,136,151)</td>
<td>0.17-0.63</td>
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<tr>
<td>24433859</td>
<td>3-amino pyridine-derived amides (51,63)</td>
<td>15-19</td>
</tr>
<tr>
<td>24405419</td>
<td>Trans-2-(pyridin-3-yl) cyclopropane carboxylic acid (39)</td>
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<td>25413783</td>
<td>Six molecules were selected as possible candidates against NAMPT for further study</td>
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<td>Cyanoguanidine-containing compounds (15)</td>
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<td>26227784</td>
<td>A non-fluorescent compound F671-0003 &amp;a fluorescent compound M049-0244</td>
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<td>26040985</td>
<td>A potent NAMPT inhibitor MS0</td>
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<td>25556090</td>
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<tr>
<td>27224875</td>
<td>(E)-N-(5-((4-((2-(1H-Indol-3-yl)ethyl)(isopropyl)amino)methyl)phenyl)amino)pentyl)-3-(pyridin-3-yl)acrylamide, 30</td>
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<td>26755394</td>
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<td>27158141</td>
<td>New NAMPT inhibitors (14)</td>
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</tr>
</tbody>
</table>

*, PubMed ID; **, Compound #; ***. The half maximal inhibitory concentration in various cancer cell lines

**New Inhibitors of NAMPT**

One promising direction to go is to develop more potent NAMPT inhibitors to increase their therapeutic efficacy while reducing IC50 to cancer cells (increasing cytotoxicity) and minimizing unwanted side effects to normal cells. Our group has reported such an attempt. Addition of a carborane to FK866 significantly increase its potency, with up to 10-fold greater anti-proliferative activity against cancer cells *in vitro* and a 100-fold increase in NAMPT inhibition [84]. One of the modified molecules with the highest potency, Compound 6, bears a meta-carborane moiety and exhibits subnanomolar IC50 values (0.31-0.41 nM) in three tested cell lines, corresponding to a 10-fold increase in potency over FK866 in DLD1, a colorectal adenocarcinoma cell line, and T47D, a human breast cancer cell line, and a 4-fold increase in A549, an adenocarcinomic human alveolar basal epithelial cell line [84], respectively. Additionally, Sadreraf K [85] has shown that the carbazole moiety of the molecule can be modified with a hydroxymethyl functional group to allow its covalent attachment to cancer-associated targeting vectors. Several new NAMPT inhibitors listed in Table 1 are either newly selected or modified from existing inhibitors based on the same principles of increasing drug potency, reducing effective dosage, and minimizing side effects. These newly developed inhibitors are extensively reviewed previously [86, 87]. Currently, there is an ongoing clinical trial on KPT-9274, a dual inhibitor of NAMPT and PAK4, in USA (NCT02702492, Clinical Trials.gov) entitled “PAK4 and NAMPT in Patients With Solid Malignancies or NHL (PANAMA)”. The trial, sponsored by Karyopharm Therapeutics, Inc (karyopharm.com) and led by Dr. Michael Kauffman, started in May 2016 and is expected to end in Dec., 2017. This is a first-in-human, multi-center, open-label clinical study with separate dose escalation and expansion phases to assess preliminary safety, tolerability, and efficacy of KPT-9274 in patients with advanced solid malignancies (including sarcoma, colon and lung cancers, etc.) or Non-Hodgkin lymphoma, for which all standard therapeutic options considered useful by the investigators have been exhausted.

**Combination Applications of NAMPT Inhibitors with Other Drugs**

Another potential therapeutic strategy for using NAMPT inhibitors to treat cancer is to pursue drug combinations. NAMPT inhibitors are found to improve the sensitivity of cancer cells to other anticancer agents [35]. The main objectives of combination chemotherapy are an increased response rate against the tumor and minimization of adverse effects of drugs without compromising efficacy of treatment. One of the combinational strategies is to apply NAMPT inhibitor with one or more of DNA damaging treatments, such as radiation, temozolomide, 5-fluouracil, fludarabina, pemetrexed, melphalan, and β-lapachone [35] to treat cancer patients. For example, GMX1777 and APO866 enhance...
the sensitivity to radiotherapy in head and neck cancer and prostate cancer through NAMPT inhibitor-mediated NAD+ depletion and a radiation therapy-mediated increase in PARP activity [88, 89]. This is in keeping with the proposed mechanism of synergy caused by a simultaneous increase in NAD+ consumption and inhibition of NAD+ regeneration. Another combinational strategy is to treat cancer patients using an NAMPT inhibitor with a targeted inhibitor such as Olaparib, TRAIL, Vorinostat, Rituxumab, Bortezomib, and FX-11(LDHA inhibitor) [35]. Zoppoli G [90] has reported a potent synergistic interaction between the NAMPT inhibitor APO866 and the apoptosis activator TRAIL in human leukemia cells. Additional combinational strategy is to administer NAMPT inhibitor with niacin to treat those tumors which lack nicotinic acid phosphoribosyltransferase 1 (NAPRT1) [35]. NAPRT1 can catalyze the conversion of niacin into NAD via the Preiss-Handler pathway [91]. NAPRT1 is expressed in the majority of normal tissues. Thus, one can apply a higher dosage of NAMPT inhibitor plus niacin to selected tumors to enhance the therapeutic potency while ameliorating their side effects on normal tissues since niacin can rescue the toxicity in normal cells engendered by NAMPT inhibitors-mediated NAD depletion [92].

Perspectives
NAMPT has drawn an ever-increasing attention in biomedical fields because of its pleiotropic physiological functions and its dysregulation implicated in a number of human diseases and conditions. A significant progress has been made in deciphering a molecular nutritional link between NAMPT and cancer. NAMPT has become an attractive target in cancer therapy. However, our understanding of the roles of NAMPT in cancer and the underlying mechanisms of action is still in its infancy. NAMPT inhibitors are far from becoming a ‘tour de force’ in cancer therapy. A number of Terra incognita in NAMPT research remain to be explored.

The present review article has highlighted the overexpression of NAMPT gene in number of cancers and a molecular nutritional link between NAMPT and cancer via NAD and obesity. Not every cancer is associated with higher expression of NAMPT and obesity. This poses a dilemma on the true causal role of NAMPT in the pathogenesis, progression, and outcomes of cancer. The NAD connection with the NAMPT enzymatic activity may explain major physiological functions of NAMPT. However, we and others have provided robust evidence supporting that NAMPT also has non-enzymatic functions [5]. What is the interplay between NAMPT’s enzymatic activity and its non-enzymatic functions and how these dual roles are weighed in the cancer warrants further investigations. There is a discrepancy in reports on the association of NAMPT with different cancers. Well-conducted epidemiological surveys or clinical studies with large sample sizes of various cancer patient populations with strict phenotyping and standardized quan-
titative NAMPT assays are needed to firmly substantiate the utility of NAMPT as a diagnostic and prognostic biomarker in cancers. The genetics of NAMPT gene in cancer is still a black box, though there are a few reports of NAMPT coding mutations associated resistance to NAMPT inhibitor treatments [35].

Although the list of small molecule inhibitors of NAMPT for cancer therapy is growing, identification of more new inhibitors or improvement of existing inhibitors is still called for to enhance the potency, lower the effective dosage and reduce the side effects. Small molecule inhibitors are a mainstay of NAMPT inhibitors but other ways such as NAMPT siRNA/shRNA, miRNA, aptamer, antisense oligonucleotide, antibody and gene knock out by CRISPR-Cas 9 system to knock down NAMPT gene expression as cancer therapeutic utilities are worth to be explored. There is a paucity of systematic study on the pharmacokinetics and pharmacodynamics of NAMPT inhibitors, either alone or in combination with other drugs. Nevertheless, it is anticipated that more improved and new NAMPT inhibitors will be developed within the next few years. In conclusion, NAMPT-based strategies hold an immense promise in management of various human cancers in the future.

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Conflict of Interests
The authors declare no conflict of interests.

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