Available online at www.sciencerepository.org

Science Repository



Mini Review

Mini-review: Current challenges in the treatment of developmentally diverse neuroendocrine like tumors: Comparison of bronchial carcinoids and neuroblastoma

Reza Bayat Mokhtari^{1,2,3*}, Narges Baluch^{4,#}, Sushil Kumar^{5,#}, Evgeniya Morgatskaya¹, Joyeeta Talukdar⁶ and Herman Yeger^{1,2,4}

¹Developmental and Stem Cell Biology, The Hospital for Sick Children Peter Gilgan Centre for Research and Learning (SICKKIDS), 686 Bay St., Rm 15.9714, Toronto, Ontario, M5G 0A4, Canada

²Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, Peter Gilgan Centre for Research and Learning (SICKKIDS), 686 Bay St., Rm 15.9714, Toronto, Ontario, M5G 0A4, Canada

³Institute of Medical Sciences, Faculty of Medicine, University of Toronto, Medical Sciences Building, 1 King's College Circle, Room 2374, Toronto, Ontario, M5S 1A8, Canada

⁴Department of Pediatrics, Queen's University, 76 Stuart St, Kingston, Ontario, K7L 2V7, Canada

⁵Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska, 68198-6025, USA

⁶Department of Bioengineering and Technology, Gauhati University Institute of Science and Technology, Guwahati, Assam, India

ARTICLE INFO

Article history:

Received: 10 September, 2018

Accepted: 26 October, 2018

Published: 18 April, 2019

Keywords:

Neuroendocrine tumor
neuroblastoma
bronchial carcinoid

ABSTRACT

Bronchial carcinoids (BC) derive from the pulmonary neuroendocrine cell system while neuroblastoma (NB) derives from the neural crest and represents the peripheral nervous system. Nevertheless, their production of serotonin and catecholamines, respectively, permits comparison as neuroendocrine tumors (NETs). BC are most often diagnosed in adults accounting for 1–5% of all invasive lung malignancies in the adults. NB, primarily a pediatric cancer, accounts for 7–8% of all childhood cancers with the highest incidence in children younger than 5 years, and frequent metastasis to liver, skin, bone, and brain. The major standard treatments for BC is surgery followed by endocrine therapy and chemotherapies. NB patients on the other hand receive treatments according to presenting stage with chemotherapies dominant and now newer immunotherapies at more advanced stages. In general BC are more indolent but when less differentiated can be aggressive portending a poorer outcome. NB in contrast often present at an advanced stage and if high stage the overall survival approaches 25% despite extensive and other therapies. Early stage diagnosis still remains a challenge since symptomatology depends on their neuroendocrine manifestation. Here we summarize the current knowledge and challenges in the management of BC and NB.

© 2018 Reza Bayat Mokhtari. Hosting by Science Repository. All rights reserved.

*Correspondence to: Reza Bayat Mokhtari; The Hospital for Sick Children Peter Gilgan Centre for Research and Learning (SICKKIDS), 686 Bay St., Rm 15.9714, Toronto, Ontario, M5G 0A4, Canada; Tel: +1 (416) 813 7654 ext. 206509; E-mail: oncoplasia@gmail.com.

Herman Yeger; The Hospital for Sick Children Peter Gilgan Centre for Research and Learning (SICKKIDS), 686 Bay St., Rm 15.9714, Toronto, Ontario, M5G 0A4, Canada; Tel: +1 (416) 813 5958; E-mail: hermie@sickkids.ca.

Introduction

Neuroendocrine tumors (NETs) arise from neuroendocrine cells in multiple tissues and organ sites. Currently, the incidence of NETs is between 3-5/100,000/year, and rising, especially for the BC subgroup, with more refined diagnoses. Thus, there is a pressing need for therapies that target these tumors [1]. NETs can be indolent or malignant and can arise in the gastrointestinal tract, lungs, pancreas and other endocrine glands. NETs have the potential to arise from neuroendocrine cells present in various organs, but the most commonly diagnosed NETs are present in the bronchopulmonary and gastrointestinal systems [2, 3]. Although NETs arise from neuroendocrine cells, they vary widely in terms of molecular features, proliferation, metastatic potential, therapeutic response, and prognosis. Some are indolent, benign and treatable, whereas others are aggressive, metastatic and have poor prognosis.

Pulmonary NETs primarily bronchial carcinoids (BC) were previously thought to be less prevalent than they actually are [4]. Pulmonary NETs make up 1-2% of all lung cancers, and a third of all NETs are pulmonary in origin. BC including typical and atypical carcinoids are well- and moderately-differentiated NETs, respectively. In general, these tumors are considered indolent and slow growing with good clinical outcomes, unless detected in late stages. Early diagnosis is usually challenging due to non-specific clinical symptoms and lack of high-level clinical evidence. Surgical resection is the main curative treatment. Treatment guidelines for non-resectable tumors are less clear. Systemic therapy with somatostatin analogue (SSA) is used in the patients with advanced disease. Recently, everolimus, an inhibitor of mammalian target of rapamycin (mTOR), has received FDA approval for treatment of advanced, non-functional, well-differentiated lung NETs [5, 6].

Neuroblastoma (NB), on the other hand, is a highly aggressive pediatric malignancy originating from neural crest. NB is the most common extra-cranial solid tumor in children, responsible for almost 13% of all childhood cancer mortality. NB presents with biological heterogeneity and diverse clinical behavior. Histological and biological characteristics are used for risk stratification of NB. Currently, patients are categorized into low-risk, intermediate-risk, and high-risk groups. Treatment options vary from observation or minimal intervention for low-risk disease to a combination of chemotherapy, radiation, surgery, and high-dose chemotherapy with autologous stem cell rescue for high-risk disease. Newer biologic and immunotherapeutic options are also frequently used in the treatment of high-risk NB. Despite the multi-modal aggressive therapy in high-risk disease, the survival rate remains low for this group of patients [7]. Therefore, newer treatment options are required to improve the disease outcome in these patients.

Typical and atypical BC are low- and intermediate-grade neuroendocrine tumors, whereas NB is an aggressive neuroendocrine tumor with the less favorable outcome. However, BC and NB share some common features that make them valuable tumor models for study and comparison of novel therapeutic approaches targeting NETs. Both carcinoids and NB arise from cells that synthesize and secrete biogenic amines. Carcinoids arise from neuroendocrine cells that synthesize serotonin from amino acid tryptophan. Carcinoids secrete serotonin, thus causing carcinoid syndrome [8]. On the other hand, NB arises mostly from cells that synthesize (from tyrosine) and secrete catecholamines, such as chromaffin cells (forming the adrenal medulla) and sympathetic ganglia. Another common feature is their response to MIBG therapy. Although not yet approved by FDA, I-131 MIBG therapy is a common therapeutic approach that has been used to target NET including NB, BC, and pheochromocytoma. Metaiodobenzylguanidine (MIBG) is an analog of guanethidine with structural similarity to neurotransmitter norepinephrine. Neuroendocrine cells take up MIBG into cells mainly through a norepinephrine transporter [6, 7]. Additionally, both NB and carcinoids share biomarkers such as synaptophysin, chromogranin-A, norepinephrine transporters and somatostatin receptors [9-11]. Synaptophysin antibody stains 100% of carcinoid and NB cells, irrespective of the site of origin and metastasis [9, 10]. Neuron-specific enolase has shown a significant correlation to tumor size at diagnosis and tumor-related death in NB and is expressed in 100% of carcinoid tumors [12, 13]. Chromogranin-A is an extensively expressed marker in both carcinoids and NB [12, 13].

Furthermore, common molecular alterations have been identified in NETs. PI3K/Akt/mTOR pathway is one of the important recognized pathways that plays a critical role in tumor growth and metastasis by promoting cell survival and cell cycle progression. Amplifications or mutations in PI3K/Akt/mTOR pathway have been identified in NETs. Although the activation of PI3K/Akt/mTOR pathway in NB has been demonstrated in different studies, the mechanisms by which the activation occurs are not clear [14]. Therapies targeting this pathway have been investigated both in BC and NB. Everolimus is such a therapy that has already received FDA approval for advanced BC [5]. mTOR inhibitors such as Temsirolimus in combination therapy have been studied in clinical trials for NB with promising results [15].

Although all NETs have the neuroendocrine origin, diversity exists in aggressiveness, therapeutic response and patient survival. Biomedical research has been striving to develop effective treatments that will maximize survival and further reduce death rates. Thus, it's essential to establish novel treatment modalities that serve to eliminate these

cancers. This review summarizes the current challenges in diagnosis and management of the two neuroendocrine tumors BC and NB.

Bronchial Carcinoids

I Characteristics

BC represents the well-differentiated subgroup of lung NET that originates from neuroendocrine Kulchitsky's cells located in the bronchial mucosa. It is characterized by an organoid arrangement of tumor cells in the form of trabecular, nesting or gyriform patterns [16]. The characteristic BC is composed of uniform small to medium-sized cells with centrally located round to oval nuclei containing fine granular chromatin and lightly eosinophilic cytoplasm [17, 18]. Based on the histological analysis and secretory behavior, BC is divided into four subtypes: typical carcinoid (TC) - a low-grade lung NET, atypical carcinoid (AC) - an intermediate grade lung NET, large cell neuroendocrine carcinoma (LCNEC), and small cell lung carcinoma (SCLC) - both high-grade lung NETs. Unlike large cell neuroendocrine carcinoma (LCNEC), and small cell lung carcinoma (SCLC), AC and TC are well-differentiated tumors [4].

Typical BC has been identified as the most common cause of Cushing's syndrome caused by ectopic secretion of corticotrophin in young adult patients [19]. The pathogenesis of BC has been the subject of considerable interest, but fewer studies have been conducted due to their originally perceived low prevalence. However, TC is still considered a low-grade malignancy rather than a benign tumor since it can involve regional lymph nodes and metastasize to distant organs in 15% of patients [20]. The 5-year survival rate of these patients is higher than 90% [20]. In contrast, the rate of metastasis and lymph node (LN) involvement in AC falls between 35-64%, and the 5-year survival rate is between 61-88% [20]. Moreover, AC tends to metastasize to contralateral LN, while LN involvement in TC is limited to ipsilateral hilum or mediastinum [21]. Carcinoid tumors were shown to cause late metastasis, and therefore, patients should be monitored closely [20].

II Epidemiology

The incidence rate of BC ranges from 0.2 to 2/100,000 population/year in Europe and the US [10, 16]. Although it is a rare type of tumor, its incidence has been increasing rapidly over the past 30 years due to increased awareness and use of special immunohistochemistry stains for diagnosis [16, 22]. BC is slightly more prevalent in white patients and in females [10]. Importantly, it is the most common primary lung cancer in the pediatric population [10]. TC is more prevalent than AC with a ratio of 8-10:1 [10]. In general, AC follows a more aggressive clinical course and presents with less favorable histological features [23]. The average

age at diagnosis is 45 years in TC and about 55 years in AC [10]. ACs are much more aggressive than TCs and have a higher instance of regional lymph node involvement by 10% (50% vs. 60%) and distant metastasis by roughly 15% (2-5% vs. 20%) [10, 22]. Furthermore, patients with AC have significantly lower 5- and 10-year survival rates [22].

III Molecular pathogenesis

Due to the inadequate response of advanced stages of this disease to the various treatments, it is important to understand its underlying molecular mechanisms to achieve a more effective treatment and a higher survival. Genetic studies show that the loss of heterozygosity (LOH) through point mutations, homozygous deletions, and aberrant promoter methylation may be linked to BC tumor development [23]. Cakir et al. reported that 22% and 55.5% of 11q13 LOH mutations had been reported in TCs and 0-73% in ACs [23]. In comparative genomic hybridization (CGH) studies, continuous copy number gains for chromosome numbers on chromosomes 5, 7 and 14, and recurrent copy number losses were observed on chromosomes 3, 11 and 22q [23, 24]. In BCs, genes that code for EGFR, hepatocyte growth factor receptor (HGFR), platelet-derived growth factor receptor B (PDGFRB), Akt1/PKB, mTOR and Kirsten rat sarcoma (KRAS) viral oncogene homolog have shown the highest rate of mutation [25-27]. In BC cells, the activation of PI3K/Akt signaling facilitates tumor cell growth and inhibits apoptosis [25]. Moreover, transcriptional up-regulation of one of the MYC proto-oncogenes has been identified in 20-30% of bronchial carcinoma cases [28]. MYC may be involved in controlling pluripotency, self-renewal, and epithelial-to-mesenchymal transition processes that are strongly implicated in cellular transformation [28]. Activation of the MYC signaling pathway leads to the activation of the oncogenic PI3K pathway which results in the deactivation of p53 [29]. Deactivating MYC is linked to an increase in apoptosis, thus proving that mutations in MYC-related genes might result in BC relapse [30]. BC tumors have also been investigated for pathways involved in epigenetic silencing via methylation of genes such as cyclin-dependent kinase inhibitor 2A (p16), adenomatous polyposis coli (APC), H-cadherin, glutathione S-transferase P1, O6-methylguanine-DNA-methyltransferase, retinoic acid receptor b-2, E-cadherin and RAS association domain family 1A (RASSF1A) [23]. The Rb/p16/cyclin D1 pathway is an extensively studied pathway in BCs where Rb expression was widely reported with loss of p16 [31] while 29% of TC cases studied by Igarashi et al (35) presented with the loss of p16. On the other hand, CD44, which is a multistructural and multifunctional cell surface molecule involved in cell proliferation differentiation and migration, angiogenesis, and cell-survival-related signaling, was found to be a prognostic indicator for neoplastic tissues and is seen inversely proportional to p53 expression in the case of NET lung tumors (Figure 1) [32, 33].

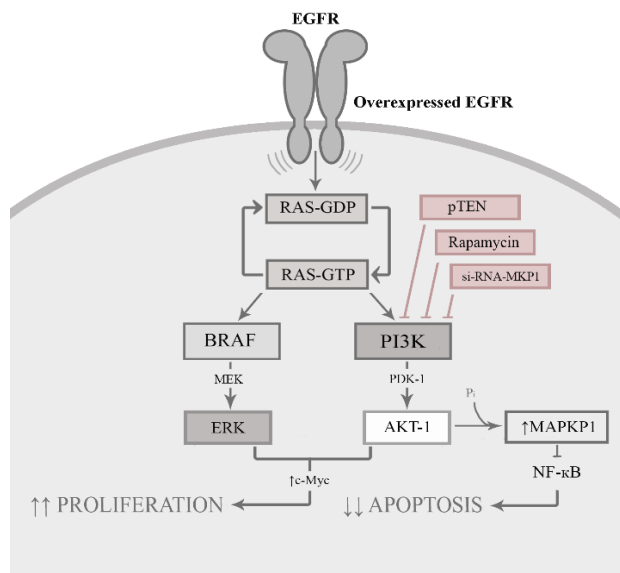


Figure 1: Bronchial Carcinoid Pathways

One of the upregulated pathways in TC and AC tumors is the phosphatidylinositol 3-kinase/protein kinase B/ mammalian target of rapamycin (PI3K/Akt/mTOR) pathway (Figure 1) [25]. In vitro model studies using BC specific cell lines NCI-H727 and NCI-H720 showed increased expression of epithelial growth factor receptor (EGFR) that in turn increased extracellular signal-regulated kinase (ERK) and Akt phosphorylation. When these tumor cells were transfected with Akt1 siRNA, their proliferation decreased [34, 35]. Moreover, the phosphorylation of Akt-1 leads to expression of mitogen-activated protein kinase phosphatase 1 (MAPK1) and results in the downregulation of NF-κB. This causes a decrease in apoptosis and an upregulation of stemness and tumorigenicity [25]. When transfected with siRNA-MKP1, PI3K inhibitor chemosensitivity is increased, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and PI3K inhibitors on cells become susceptible to cisplatin, a chemotherapeutic drug [34]. As a result, the Ras pathway downstream distortion reduces the proliferation rate of in-situ cells and causes them to undergo apoptosis [36].

IV. Effects of standard therapy on BC-cell survival, apoptosis and differentiation

Surgical resection remains the gold standard therapy for BC. Early stage BC is usually successfully treated with resection, which aims to completely remove the tumor while preserving healthy lung tissue [22]. The type and extent of surgery depend on the tumor type, location, and size. Surgery can be considered as part of the multimodal management for metastatic disease if there are limited sites of disease where radical surgery with the intent of cure is possible [10]. Curative or palliative

surgery is considered in cases with liver metastasis if >90% of the tumor can be resected. Successful resection of liver metastasis can improve the 5-year overall survival rate to over 70% [37]. However, curative surgery is not possible in the presence of right heart insufficiency, unresectable lymph node and extra-abdominal metastases and diffuse or unresectable peritoneal carcinomatosis [38].

Medical therapy is incorporated in the management of BC particularly in advanced disease. In addition to tumor growth, medical therapy targets hormone-related symptoms in BC [39]. Up to 30% of patients with advanced BC suffer from hormone-related symptoms [10]. Compared to digestive system NETs, hormonal secretions in BC are more diverse; therefore, optimal control of hormone-related symptoms can significantly improve patients' quality of life, particularly in metastatic or advanced disease. Carcinoid syndrome is the most common hormone disturbance in BC. The standard treatment for symptom control in carcinoid syndrome is somatostatin analogs (SA) [40]. Somatostatin is a peptide that binds to G-protein-coupled receptors on the cell surface to exert its inhibitory effects. Although somatostatin inhibits NET-induced hormone release and cancer cell growth, its clinical use is limited by its short half-life; this has led to the development of drugs with a longer lifespan. Octreotide, an FDA-approved SA, is indicated in the management of severe symptoms of carcinoid syndrome such as profuse diarrhea and flushing [40]. Lanreotide is another FDA-approved SA used for gastroenteropancreatic (GEP) NETs in 2014. Lanreotide improves progression-free survival in unresectable non-functional, well, and moderately-differentiated GEP-NET with local involvement or distant metastasis [41]. However, a similar effect by lanreotide on BC has not yet been documented. Chemoradiation is recommended for locally advanced non-resectable AC, and definitive radiation without chemotherapy for advanced non-resectable TC; however, the ability of tumors to develop resistance to treatment and the short half-life of chemotherapeutic drugs makes it difficult to properly treat BC. Although chemotherapy is recommended for locally advanced AC, it is considered unreliable in many cases [40].

V. Potential options for treatment of BC

Anticancer drugs and carcinogens might play a main role in causing cancer relapse by causing resistance to apoptosis one of the hallmarks of cancer. In many cases, relapse occurs due to the activation/deactivation of several signaling pathways [42]. Previous study showed that vascular endothelial growth factor (VEGF) and its receptors have been found to be over-expressed in carcinoid cancer cells[43]. However, anti-VEGF therapies such as sunitinib had not demonstrated similar clinical trial success in carcinoids as in less differentiated NETs[44-46]. In addition, Zhang et al. reported that BCs exhibit mutations in mTOR pathway distinct from lung cancer[47]. In a Phase-III randomized placebo-

controlled clinical trial in carcinoid patients, mTOR inhibitor everolimus in combination with octreotide was associated with higher PFS compared to placebo and octreotide, though the difference was not statistically significant. Moreover, mTOR and IGF-1 inhibition in specific BC cell lines significantly reduced chromogranin A and VEGF secretion [48]. Studies in our laboratory, with a focus on adjuvant systemic therapy identified a combination of drugs that would effectively target BC tumors. Acetazolamide (AZ), a pan-carbonic anhydrase inhibitor, was shown to significantly potentiate the anti-tumor effects of sulforaphane (SFN, a natural isothiocyanate with HDACi activity) in BC cell lines [49].

Valproic acid or suberoyl bis-hydroxamic acid (SBHA), a synthetic histone deacetylase inhibitor, was shown to decrease proliferation and up-regulation of Notch-1, which is known to decrease cell proliferation. Notch-1 upregulation leads to p53 expression and decreased BC cell growth [50, 51]. However, further clinical trials are required to improve the chances of survival of BC patients using adjuvant therapy, as it is an under-researched field and its efficiency in treating BC cannot be accurately estimated or optimized [22].

Neuroblastoma

Neuroblastoma (NB) is the most common pediatric solid tumor: over 650 cases present annually in North America alone [52]. NB is a highly aggressive cancer derived from the neural crest and is particularly challenging to treat in children older than 4 years. The likelihood of surviving NB is inversely related to the patient's age. The International Neuroblastoma Staging System (INSS) was first introduced in 1988 and is widely used for the clinical purposes [53]. There are 4 stages of NB in INSS: 1, 2A, 2B, 3, 4 and 4S. These stages are based on surgical excision, lymph node involvement and metastatic sites [54, 55]. Stage 4S disease is also called special neuroblastoma. It is only diagnosed in children younger than 12 months. Although the disease has spread to liver, bone marrow and skin, patients have very good prognosis [54, 55]. The tumor in stage I disease can be resected completely. Stages 2 and 3 are characterized by partially resected regional tumors. Stage 4 is associated with the distant metastatic spread of the disease to bone, bone marrow and central nervous system (CNS). If detected in the early stages, surgery provides 95% cure rate. This number decreases significantly to 60% with stage 3 tumors. Stage 4 disease is associated with poor prognosis despite intensive multimodal therapy [54, 55, 56]. Moreover, NB can be sporadic or non-familial in origin. It primarily resides in the abdominal region from which it can metastasize into lymph nodes, the liver, intracranial and orbital sites, as well as the central nervous system [57-60]. Whereas low-grade NB can be treated by surgery alone, advanced and metastatic NBs are treated by multimodal therapy consisting of surgery, radiotherapy, and chemotherapy [60]. The

dangers of NB primarily arise from its latent manifestation as a highly aggressive metastatic cancer which is unresponsive to standard chemotherapy [61].

I Characteristics

NB is derived from the neuronal ganglia of the primitive neural crest which forms the peripheral sympathetic nervous system [62]. It is a developmental malignancy that occurs when neuronal structures develop from the ventrolateral neural crest and migrate away from it during embryogenesis [63]. The adrenal gland, sympathetic ganglia, and parasympathetic ganglia are the most common sites affected by NB [63, 64]. Being a pediatric cancer, NB exhibits both biological and clinical heterogeneous complexity as compared to adult tumors. The extensive clinical and pathologic heterogeneity of this malignancy reflects the unique developmental biology of the neural crest [60, 65, 66]. NB is composed of small, monomorphous cells with solid hyperchromatic nuclei and scant cytoplasm. Peripheral neuroblastic tumors (pNTs) stain positive for neural markers that differentiate them from other small blue cell tumors [54]. Three different morphological categories of pNTs are described:

I. NB (Schwannian-stroma poor): the NB tumor is composed of nests of neuroblastic cells

separated by stromal septa with almost no Schwannian proliferation. The Schwannian-stroma poor NB is further divided into three subtypes known as undifferentiated, poorly differentiated (Pseudorosette) and differentiating (Ganglioneuroma) NB.

II. Intermixed Ganglioneuroblastoma (Schwannian stroma-rich): This type of NB is characterized by scattered groups of neuroblastic cells in ganglioneuromatous stroma. Neuroblastic cells are in different stages of maturation.

III. Nodular Ganglioneuroblastoma (Schwannian stroma – rich/stroma dominant and stroma-poor): this type of tumor is composed of visible neuroblastic nodules, which represent the stroma poor, aggressive component of tumor. The other component of tumor is nonaggressive intermixed ganglioneuroblastoma and ganglioneuroma [54].

Unlike in adult cancers, there are very few recurrent mutations in NB; however, these mutations have diverse molecular stages [63]. Epithelial-to-mesenchymal Transition (EMT) is the central component for maturation of the neural crest. NB-initiating cells or cancer stem cells (CSCs) might initiate the distinct tumor phenotypes by using this EMT mechanism in various developmental stages which results from various types of mutations [63, 67]. High cellular heterogeneity is characteristic of NB; NB tumors have no static cellular hierarchy, and the presence of CSC populations allows NB cells to transdifferentiate into various cell

lineages which lack NB cell markers [68]. Because of this, responses of tumors to treatment are difficult to predict [68].

II Epidemiology

NB constitutes 7-8% of all childhood cancers with the highest incidence in children younger than 5 years and is the most common cancer diagnosed in children under the age of one [69]. The prevalence of NB is approximately 1 case per 7,000 live births. About 37% of cases are diagnosed in infancy, and 90% are diagnosed in children under 5 years old [70]. Although there is no racial difference in the overall incidence of NB, African-American children are more likely to present with high-risk disease and ultimately have a worse prognosis [70, 71]. Clinical presentation of NB can vary from localized to widely spread metastatic disease, however, the one descended from neural crest cells in the form of malignant neuroblasts is the most common [69]. The undifferentiated sympathoadrenal lineage cells differentiate in the neural crest region to form the peripheral sympathetic nervous system. This differentiation leads to the formation of NB tumors in various regions including the adrenal medulla, abdomen and sympathetic ganglia [60]. Symptoms of NB arise when tumors reach a great enough mass to interfere with regular body function or metastasize to a different region in the body. Common sites of NB metastasis are the liver, skin, bone, and brain resulting in survival rates less than 40% despite intensive treatment [72, 73].

III Molecular Pathogenesis

One of the malignancy-inducing mutations is that of the MYCN oncogene located on chromosome 2 [74]. MYCN is a major culprit that contributes to the development of aggressive NB and is, therefore, a common NB marker [75]. Studies show that MYCN overexpression occurs in 25% of NB cases, and is partly responsible for tumorigenic and anti-angiogenic qualities of the tumors [75]. Clinical approaches where MYCN has been targeted along with downstream pathways such as MDM2 and mTOR are not always adequate or sufficient since many high-risk cases of NB show minimal MYCN expression; because of this, additional pathways that are independent of MYCN signaling such as RAS-MAPK, NB-RAS viral oncogene homolog (NRAS), and neurofibromin 1 (NF1) must be targeted for effective NB treatment [63, 76, 77]. Currently, the receptor tyrosine kinase (RTK) anaplastic lymphoma kinase (ALK), a transcriptional target of MYCN and an important stem cell function regulator has been found to promote tumorigenesis in several NB case studies. Compounds that can potentially target and disrupt ALK are already under investigation in clinical trials [78, 79]. Targeting proteins involved in MYCN regulation may serve as an effective therapeutic strategy, particularly in high-risk

NB patients with MYCN overexpression [78, 79]. Das et al. developed a novel strategy that uses DNA methylation analysis and miRNA expression to identify a panel of epigenetically regulated miRNAs that contribute to NB pathogenesis [80]. Long noncoding RNA—NBAT-1 (NB-Associated Transcript 1) were found to epigenetically down-regulate the tumorigenic factors and promote differentiation of NB tumor cells [81]. The involvement of long non-coding RNAs (lncRNAs), alternative splicing programs, fragile sites and genome-wide methylation has been emphasizing the importance of the developmental context of NB (Figure 2), [60, 81-83].

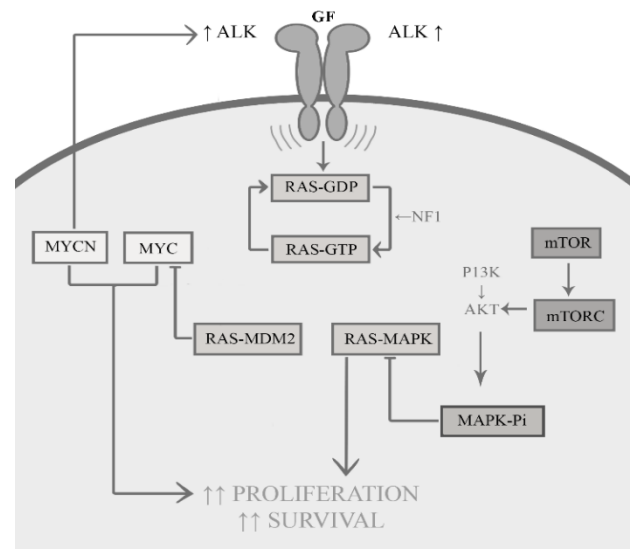


Figure 2: Neuroblastoma Pathways

IV Effects of Standard Therapy on NB-Cell Survival, Apoptosis, and Differentiation

Due to new treatment advancements, the survival rate of NB patients has increased from 86% to 95% in children under a year old, and from 34% to 68% for between the ages of 1 and 14 years [60]. The Children's Oncology Group (COG) has defined three risk groups for NB: low-risk, intermediate-risk and high-risk groups based on the International Neuroblastoma Staging System (INSS) [84, 85]. The standard of therapy for the low-risk disease is either observation or surgical resection. These patients enjoy a 5-year outcome after surgery (OS) of 97% [86]. Patients with intermediate-risk disease usually receive chemotherapy prior to resection. Duration and amount of chemotherapy are determined by tumor biological and clinical risk factors and response to therapy. The 3-year OS for these patients is around 96% [87]. High-risk neuroblastoma (HR-NB) disease is associated with a poor prognosis despite multimodal treatment. Different treatment options that are indicated for HR-NB include surgical resection, chemotherapy, radiation therapy, immunotherapy, hematopoietic stem cell transplantation, and differentiation therapy. The OS for these patients remains low at 40-50% [88-90]. Different treatment options that are indicated for HR-NB include surgical resection, chemotherapy, radiation therapy, immunotherapy, hematopoietic stem cell transplantation, and differentiation therapy. Chemotherapy for patients with HR-NB includes intensive induction chemotherapy, followed by myeloablative

consolidation chemotherapy with stem cell rescue. Surgical resection of tumor and bulky metastasis is usually attempted after patient has received a few cycles of chemotherapy [91]. Some studies have shown better outcomes if at least 4 cycles of chemotherapy have been delivered prior to surgical resection[92]. Autologous bone marrow transplant (BMT) and differentiation therapy using 13-cis-retinoic acid are associated with improved event free survival (EFS) compared to chemotherapy alone and therefore, have been included in most of the HR-NB treatment protocols[88]. However, it's not completely clear whether these treatment options provide long term survival advantage[88]. After stem cell transplant, external beam radiation therapy is administered to the primary tumor site and metastatic sites [93]. MIBG radiotherapy is another method of irradiation in HR-NB [94]. Short- and long-term side effects of radiation therapy can be debilitating in pediatric patients. HR-NB remains a therapeutic challenge for clinicians. The OS for patients with HR-NB is still low at 40-50% despite intensive treatment that is associated with significant side effects[59, 93].

In the present decade, immunotherapy has shown promise in treating high-risk NB. Anti-GD2 antibody dinutuximab has been recently approved by US FDA as first-line therapy for high-risk neuroblastoma, based upon clinical trial [95] in which immunotherapy with dinutuximab, GM-CSF, and interleukin-2 was significantly superior to standard therapy in terms of EFS and OS [96]. Dinutuximab has shown significant anti-tumor activity in Phase-II trial in combination with irinotecan, and temozolomide[15]. Here 53% patients achieved objective response compared to 6% response in patients treated with the combination irinotecan, temozolomide and temsirolimus. Recently, second-generation anti-GD2 antibody 3F8 with GM-CSF has shown promising results in treating MYCN amplified NB, with 72% and 84% 5-year EFS and OS respectively[97]. Inhibition of ALK has been proposed as a potential target treatment (71, 72, 73) ALK (Anaplastic lymphoma kinase) inhibitor ceritinib has been reported to cause complete remission in all metastatic sites in an adolescent boy [98]. ASP3026 (a second-generation ALK-inhibitor) had shown the clinical response in neuroblastoma [99]. Another ALK inhibitor PF-06463922 has shown significant efficacy in pre-clinical models of crizotinib-resistant neuroblastoma [100].

V Potential options for treatment of NB

HDACi are currently being evaluated in NB clinical trials with promising results because HDACs play a vital role in carcinogenesis and their inhibition can prove crucial in preventing it [101-106]. HDACi might hence be more effective other agents since they target many different steps of tumor cell hypoxic response that occurs in a staggering amount of cancers [107]. The antitumor potential of HDACi in combination with a carbonic anhydrase inhibitor is significantly increased [107]. A study conducted by Mokhtari et al (102) showed that nanomolar concentrations of pyridylmethyl-N-(4-[(2-aminophenyl)-carbamoyl]-benzyl)-carbamate (MS-275) alone significantly reduced the putative CSC fraction of NB cell lines leading to reduction in initial tumorigenicity and potential elimination of the NB/CSC fraction. Moreover, they showed that the anti-tumor potential of MS-275 increased when combined with AZ, which confirmed that AZ increases

the effect of the MS-275 (HDACi) on NB both in vitro and in vivo [101, 107, 108].

Antiangiogenic strategies such as metronomic chemotherapy and anti-VEGF therapy have shown anti-tumor activity in pre-clinical and clinical trials. Metronomic chemotherapy involves administration of low-dose cytotoxic agents for the prolonged duration. Metronomic chemotherapy is effective as maintenance therapy [109]. Since metronomic chemotherapy utilizes off-patent chemotherapeutics, it is an effective and affordable option for treating neuroblastoma, especially in low-income countries [110, 111].

Albumin-bound nanoparticles of paclitaxel (nab-paclitaxel) is being investigated for several pediatric cancers including neuroblastoma. In the preclinical trial, nab-paclitaxel has demonstrated significant anti-tumor activity in neuroblastoma models and achieved higher intratumoral accumulation of paclitaxel than conventional chremophore based formulation [112]. Phase-II trial of nab-paclitaxel in patients with refractory/recurrent neuroblastoma is underway [113].

Although currently, the survival rate in high-risk NB is around 50%, novel strategies mentioned above, such as immunotherapy, epigenetic modulators, nanoparticulate dosage forms and targeted therapy are expected to enhance the survival and quality of life in NB patients in the near future.

Discussion

NETs are complex tumors that require a multidisciplinary approach and long-term follow-up [1, 2, 4]. These tumors have been found to be clinically and pathologically heterogeneous, present commonly with only mild and vague symptoms which delay the diagnosis for years thus leading to disease prognosis [10, 84, 114-116]. Current therapy for the NETs including BC and NB has emerged via integration of tumor biology and therapeutics. Further improvements in our understanding of the biology of these tumors will facilitate the development of new and more effective therapies.

Treatments with individual therapeutic compounds have only moderately improved progression-free survival of cancer patients. Because of their discrete targeted age groups, aggressiveness levels, and levels of stemness, BC and NB are very well-suited NETs as models for new drug development: NB is an aggressive pediatric cancer that is difficult to target due to its easily up-regulatable stemness state while BC is less aggressive, more differentiated, and mostly affects adults [60, 117, 118]. Conventional therapies are only partially successful and are thus rarely reliable, in part due to the ability of tumors to develop drug resistance and survive treatment [108, 118]. Furthermore, because many therapeutic agents used today are highly cytotoxic, their unsuccessful attempts to treat resistant cells only damage healthy tissues and severely weaken patients [108]. Therefore, research to find a different therapeutic approach that can provide new and less toxic options for cancer targeting and that can solve the drug resistance problem should be encouraged.

Extensive cancer research over the past decade provided a lot of evidence that tumors interact with surrounding cells, molecules and vascular and lymphatic networks in the tissue in which they occur [119].

The relationship between tumor cells and noncancerous cells and proteins is referred to as the tumor microenvironment (TME), and these components interact and regulate one another. The effect of the TME on tumor progression is varied and may prevent or promote carcinogenesis [119]. The metastatic recurrence for both NB and BC has been thought to be due to CSCs, members of the TME that are able to transdifferentiate between lineages and lead to metastasis and drug resistance [120, 121]. The intermediate filament protein nestin and the transmembrane protein adenosine triphosphate-binding cassette 2 (ABCG2) have been described as the neural precursor markers used to describe CSCs in NB as they can be involved in preventing chemotherapeutic drugs from penetrating cells and may lead to chemotherapeutic resistance [119, 122]. Other CSC markers studied in BC and NB are CD144 and EpCam [123, 124]. CD144+/EpCam+ CSCs of BC also exhibited the characteristics of multipotent stem cells [124]. Targeting the interaction between NB/BC cancer-initiating cells and the elements of the tumor microenvironment and their interactions might provide novel therapeutic targets for these therapeutically-challenging diseases [119].

In our previous work on pediatric cancer [108] and in our present work on bronchial carcinoid (manuscript in preparation), we identified potent anti-tumor agents such histone HDACi that could reduce the CSC populations in cancers in order to force differentiation in stem-like tumor cells, and thus make them more readily targetable with anticancer drugs [108]. Furthermore, HDACIs are potent in increasing sensitivity to therapeutics [108]. Under hypoxic conditions, which are known to force stem-like states in CSCs, HDACIs has been shown to modulate the HIF-1 α -mediated pathway by targeting HIF-1 α and thus preventing stemness, self-renewal, and metastatic properties of tumor cells in both BC and NB [125]. Mokhtari et al. have also explored the direct effects of the SFN on BC[49]. SFN counters hypoxic conditions in which CSCs thrive, reduces CSC numbers and downregulates stemness of tumor cells [49, 121].

Conclusion

As illustrated in figures 1 and 2, NB and BC are NETs capable of resisting therapy and result in poor prognosis and even relapse in seemingly cured patients. However, adjuvant molecular therapy might be a new initiative in fighting these NETs. Identifying novel tumor targets and harnessing of cell-mediated immunotherapy may generate effective therapeutic approaches. Despite new research in NB therapy, little effective anticancer drugs exist for this disease because of its aggressiveness and transdifferentiation potential [63, 75]. More research into potent anti-NB drugs and combination therapy is required to adequately address the therapeutic challenges of this highly aggressive pediatric cancer. Our studies using the combination of AZ with SFN and MS-275 has yielded evidence for increased value in terms of the management of BC and NB tumor progression and a possible decrease in metastasis. Thus, with viable in vitro and in vivo evidence this adjuvant therapy might be considered for vigorous clinical study in both NB and BC tumors.

List of abbreviations

BC: Bronchial carcinoid; NB: neuroblastoma; NET: neuroendocrine tumors; TC: typical carcinoid; AC: atypical carcinoid; LCNEC: large cell neuroendocrine carcinoma; SCLC: small cell lung carcinoma;

LCNEC: large cell neuroendocrine carcinoma; LN: lymph node; PI3K/Akt/mTOR: phosphatidylinositol 3-kinase/protein kinase B/ mammalian target of rapamycin; EGFR: epithelial growth factor receptor; ERK: extracellular signal-regulated kinase; MAPK1: mitogen-activated protein kinase phosphatase 1; NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells; LOH: loss of heterozygosity; CGH: comparative genomic hybridization; MET: hepatocyte growth factor receptor; PDGFRB: platelet-derived growth factor receptor B; KRAS: Kirsten rat sarcoma; APC: adenomatous polyposis coli; RAS association domain family 1A: RASSF1A; somatostatin analogs: SA; gastroenteropancreatic: GEP; acetazolamide: AZ; sulforaphane: SFN; vascular endothelial growth factor: VEGF; suberoyl bis-hydroxamic acid: SBHA; receptor tyrosine kinase: RTK; anaplastic lymphoma kinase: ALK; NB-RAS viral oncogene homolog: NRAS; Children's Oncology Group: COG; outcome after surgery: OS; High-risk neuroblastoma: HR-NB

Conflict of Interest Statement

None declared.

Authors' Contributions

RBM, NB, SK wrote the manuscript. BD, HY review, and edit the manuscript.

Acknowledgment

This work is supported by supported by a grant from the Cancer Research Society (CRS) joint with the Canadian Neuroendocrine Tumor Society (CNETS).

REFERENCES

1. Hallet J, Law CH, Cukier M, Saskin R, Liu N, et al. (2015) Exploring the rising incidence of neuroendocrine tumors: a population-based analysis of epidemiology, metastatic presentation, and outcomes. *Cancer* 121: 589-597.
2. Horsch D, Schmid KW, Anlauf M, Darwiche K, Denecke T, et al. (2014) Neuroendocrine tumors of the bronchopulmonary system (typical and atypical carcinoid tumors): current strategies in diagnosis and treatment. Conclusions of an expert meeting February 2011 in Weimar, Germany. *Oncol Res Treat* 37: 266-276.
3. Salyers WJ, Vega KJ, Munoz JC, Trotman BW, Tanev SS (2014) Neuroendocrine tumors of the gastrointestinal tract: Case reports and literature review. *World J Gastrointest Oncol* 6: 301-310.
4. Bartsch DK, Albers MB, Lopez CL, Apitzsch JC, Walthers EM, et al. (2016) Bronchopulmonary Neuroendocrine Neoplasms and Their Precursor Lesions in Multiple Endocrine Neoplasia Type 1. *Neuroendocrinology* 103: 240-247.
5. Ramirez RA, Chauhan A, Gimenez J, Thomas KEH, Kokodis I, et al. (2017) Management of pulmonary neuroendocrine tumors. *Rev Endocr Metab Disord* 18: 433-442.

6. Sadowski SM, Christ E, Bedat B, Kollar A, Karenovics W, et al. (2018) Nationwide multicenter study on the management of pulmonary neuroendocrine (carcinoid) tumors. *Endocr Connect* 7: 8-15.
7. Nakagawara A LY, Lzumi H, Muramori K, Inada H, Nishi M (2018) Neuroblastoma. *Japanese Journal of Clinical Oncology* 48: 214-241.
8. Hendifar AE, Marchevsky AM, Tuli R (2016) Neuroendocrine Tumors of the Lung: Current Challenges and Advances in the Diagnosis and Management of Well-Differentiated Disease. *J Thorac Oncol*.
9. Park SJ, Park CJ, Kim S, Jang S, Chi HS, et al. (2010) Detection of bone marrow metastases of neuroblastoma with immunohistochemical staining of CD56, chromogranin A, and synaptophysin. *Appl Immunohistochem Mol Morphol* 18: 348-352.
10. Caplin ME, Baudin E, Ferolla P, Filosso P, Garcia-Yuste M, et al. (2015) Pulmonary neuroendocrine (carcinoid) tumors: European Neuroendocrine Tumor Society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids. *Ann Oncol* 26: 1604-1620.
11. Streby KA, Shah N, Ranalli MA, Kunkler A, Cripe TP (2015) Nothing but NET: a review of norepinephrine transporter expression and efficacy of 131I-mIBG therapy. *Pediatr Blood Cancer* 62: 5-11.
12. Georgantzi K, Skoldenberg EG, Stridsberg M, Kogner P, Jakobson A, et al. (2018) Chromogranin A and neuron-specific enolase in neuroblastoma: Correlation to stage and prognostic factors. *Pediatr Hematol Oncol* 1-10.
13. Mjones P, Sagatun L, Nordrum IS, Waldum HL (2017) Neuron-Specific Enolase as an Immunohistochemical Marker Is Better Than Its Reputation. *J Histochem Cytochem* 65: 687-703.
14. King D, Yeomanson D, Bryant HE (2015) PI3King the lock: targeting the PI3K/Akt/mTOR pathway as a novel therapeutic strategy in neuroblastoma. *J Pediatr Hematol Oncol* 37: 245-251.
15. Mody R, Naranjo A, Van Ryn C, Yu AL, London WB, et al. (2017) Irinotecan-temozolomide with temsirolimus or dinutuximab in children with refractory or relapsed neuroblastoma (COG ANBL1221): an open-label, randomised, phase 2 trial. *Lancet Oncol* 18: 946-957.
16. Zeichner SB, Cusnir M, Francavilla M, Hirzel A (2011) Typical bronchial carcinoid metastasizing to the brain: a case presentation. *Case Rep Oncol* 4: 602-610.
17. Nozieres C, Chardon L, Goichot B, Borson-Chazot F, Hervieu V, et al. (2016) Neuroendocrine tumors producing calcitonin: characteristics, prognosis and potential interest of calcitonin monitoring during follow-up. *Eur J Endocrinol* 174: 335-341.
18. Orakwe OI, Ukekwe FI, Okwulehie V, Iloanus N, Aghaji M (2015) Bronchial carcinoid tumors: A rare malignant tumor. *Niger J Clin Pract* 18: 698-701.
19. Witek P, Witek J, Zielinski G, Podgajny Z, Kaminski G (2015) Ectopic Cushing's syndrome in light of modern diagnostic techniques and treatment options. *Neuro Endocrinol Lett* 36: 201-208.
20. Pusceddu S, Lo Russo G, Macerelli M, Proto C, Vitali M, et al. (2016) Diagnosis and management of typical and atypical lung carcinoids. *Crit Rev Oncol Hematol* 100: 167-176.
21. Teran MD, Brock MV (2014) Staging lymph node metastases from lung cancer in the mediastinum. *J Thorac Dis* 6: 230-236.
22. Wolin EM (2015) Challenges in the Diagnosis and Management of Well-Differentiated Neuroendocrine Tumors of the Lung (Typical and Atypical Carcinoid): Current Status and Future Considerations. *Oncologist* 20: 1123-1131.
23. Cakir M, Grossman A (2011) The molecular pathogenesis and management of bronchial carcinoids. *Expert Opin Ther Targets* 15: 457-491.
24. Zhang Z, Wang M (2017) PI3K/AKT/mTOR pathway in pulmonary carcinoid tumours. *Oncol Lett* 14: 1373-1378.
25. Cortes-Sempere M, Chattopadhyay S, Rovira A, Rodriguez-Fanjul V, Belda-Iniesta C, Tapia M, et al. (2009) MKP1 repression is required for the chemosensitizing effects of NF-kappaB and PI3K inhibitors to cisplatin in non-small cell lung cancer. *Cancer Lett* 286: 206-216.
26. Pitt SC, Chen H, Kunnimalaiyaan M (2009) Phosphatidylinositol 3-kinase-Akt signaling in pulmonary carcinoid cells. *J Am Coll Surg* 209: 82-88.
27. Valentino JD, Li J, Zaytseva YY, Mustain WC, Elliott VA, et al. (2014) Cotargeting the PI3K and RAS pathways for the treatment of neuroendocrine tumors. *Clin Cancer Res* 20: 1212-1222.
28. Voortman J, Lee JH, Killian JK, Suuriniemi M, Wang Y, et al. (2010) Array comparative genomic hybridization-based characterization of genetic alterations in pulmonary neuroendocrine tumors. *Proc Natl Acad Sci USA* 107: 13040-13045.
29. Vollbrecht C, Werner R, Walter RF, Christoph DC, Heukamp LC, et al. (2015) Mutational analysis of pulmonary tumours with neuroendocrine features using targeted massive parallel sequencing: a comparison of a neglected tumour group. *Br J Cancer* 113: 1704-1711.
30. Ying M, Zhu XX, Zhao Y, Li DH, Chen LH (2015) KRAS Mutation as a Biomarker for Survival in Patients with Non-Small Cell Lung Cancer, A Meta-Analysis of 12 Randomized Trials. *Asian Pac J Cancer Prev* 16: 4439-4445.
31. Chappell J, Dalton S (2013) Roles for MYC in the establishment and maintenance of pluripotency. *Cold Spring Harb Perspect Med*.
32. Semenova EA, Nagel R, Berns A (2015) Origins, genetic landscape, and emerging therapies of small cell lung cancer. *Genes Dev* 29: 1447-1462.
33. Leone A, Roca MS, Ciardiello C, Terranova-Barberio M, Vitagliano C, (2015) Vorinostat synergizes with EGFR inhibitors in NSCLC cells by increasing ROS via up-regulation of the major mitochondrial porin VDAC1 and modulation of the c-Myc-NRF2-KEAP1 pathway. *Free Radic Biol Med* 89: 287-299.
34. Li AF, Li AC, Tsay SH, Li WY, Liang WY, et al. (2008) Alterations in the p16INK4a/cyclin D1/RB pathway in gastrointestinal tract endocrine tumors. *Am J Clin Pathol* 130: 535-542.
35. Naranjo Gomez JM, Bernal JF, Arranz PG, Fernandez SL, Roman JJ (2014) Alterations in the expression of p53, KLF4, and p21 in neuroendocrine lung tumors. *Arch Pathol Lab Med* 138: 936-942.
36. Swarts DR, Henfling ME, Van Neste L, van Suylen RJ, Dingemans AM, et al. (2013) CD44 and OTP are strong prognostic markers for

- pulmonary carcinoids. *Clinical cancer research : an official journal of the American Association for Cancer Research* 19: 2197-2207.
37. Glazer ES, Tseng JF, Al-Refaie W, Solorzano CC, Liu P, et al. (2010) Long-term survival after surgical management of neuroendocrine hepatic metastases. *HPB (Oxford)* 12: 427-433.
 38. Pavel M, Baudin E, Couvelard A, Krenning E, Oberg K, et al. (2012) ENETS Consensus Guidelines for the management of patients with liver and other distant metastases from neuroendocrine neoplasms of foregut, midgut, hindgut, and unknown primary. *Neuroendocrinology* 95: 157-176.
 39. Phan AT, Oberg K, Choi J, Harrison LH, Jr Hassan MM, et al. (2010) NANETS consensus guideline for the diagnosis and management of neuroendocrine tumors: well-differentiated neuroendocrine tumors of the thorax (includes lung and thymus). *Pancreas* 39: 784-798.
 40. Wolin EM (2012) The expanding role of somatostatin analogs in the management of neuroendocrine tumors. *Gastrointest Cancer Res* 5: 161-168.
 41. Caplin ME, Pavel M, Ruzsiewicz P (2014) Lanreotide in metastatic enteropancreatic neuroendocrine tumors. *N Engl J Med* 371: 1556-1557.
 42. Pavel ME, Hainsworth JD, Baudin E, Peeters M, Horsch D, et al. (2011) Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. *Lancet* 378: 2005-2012.
 43. Silva SR, Bowen KA, Rychahou PG, Jackson LN, Weiss HL, et al. Evers BM VEGFR-2 expression in carcinoid cancer cells and its role in tumor growth and metastasis. *International journal of cancer* 128: 1045-1056.
 44. Zhang J, Jia Z, Li Q, Wang L, Rashid A, et al. (2007) Elevated expression of vascular endothelial growth factor correlates with increased angiogenesis and decreased progression-free survival among patients with low-grade neuroendocrine tumors. *Cancer* 109: 1478-1486.
 45. Raj N, Reidy-Lagunes D (2014) Current clinical trials of targeted agents for well-differentiated neuroendocrine tumors. *Pancreas* 43: 1185-1189.
 46. Bajetta E, Guadalupe V, Procopio G (2009) Activity of sunitinib in patients with advanced neuroendocrine tumors. *J Clin Oncol* 27: 319-320.
 47. Cheng H, Shcherba M, Pendurti G, Liang Y, Piperdi B, et al. (2014) Targeting the PI3K/AKT/mTOR pathway: potential for lung cancer treatment. *Lung Cancer Manag* 3: 67-75.
 48. Grozinsky-Glasberg S, Franchi G, Teng M, Leontiou CA, Ribeiro de Oliveira A, et al. (2008) Octreotide and the mTOR inhibitor RAD001 (everolimus) block proliferation and interact with the Akt-mTOR-p70S6K pathway in a neuro-endocrine tumour cell Line. *Neuroendocrinology* 87: 168-181.
 49. Mokhtari RB, Kumar S, Islam SS, Yazdanpanah M, Adeli K, et al. (2013) Combination of carbonic anhydrase inhibitor, acetazolamide, and sulforaphane, reduces the viability and growth of bronchial carcinoid cell lines. *BMC Cancer* 13: 378.
 50. Sharma S, Taliyan R (2015) Synergistic effects of GSK-3beta and HDAC inhibitors in intracerebroventricular streptozotocin-induced cognitive deficits in rats. *Naunyn Schmiedebergs Arch Pharmacol* 388: 337-349.
 51. Adler JT, Hottinger DG, Kunnimalaiyaan M, Chen H (2009) Combination therapy with histone deacetylase inhibitors and lithium chloride: a novel treatment for carcinoid tumors. *Ann Surg Oncol* 16: 481-486.
 52. Artal Cortes A, Calera Urquiza L, Hernando Cubero J (2015) Adjuvant chemotherapy in non-small cell lung cancer: state-of-the-art. *Transl Lung Cancer Res* 4: 191-197.
 53. Navalkele P, O Dorisio MS, O Dorisio TM, Zamba GK, Lynch CF (2011) Incidence, survival, and prevalence of neuroendocrine tumors versus neuroblastoma in children and young adults: nine standard SEER registries, 1975-2006. *Pediatr Blood Cancer* 56: 50-57.
 54. Morgenstern DA, London WB, Stephens D, Volchenboum SL, Hero B, et al. (2014) Metastatic neuroblastoma confined to distant lymph nodes (stage 4N) predicts outcome in patients with stage 4 disease: A study from the International Neuroblastoma Risk Group Database. *J Clin Oncol* 32: 1228-1235.
 55. Maman S, Edry-Botzer L, Sagi-Assif O, Meshel T, Yuan W, et al. (2013) The metastatic microenvironment: lung-derived factors control the viability of neuroblastoma lung metastasis. *Int J Cancer* 133: 2296-2306.
 56. Lee S, Qiao J, Paul P, O'Connor KL, Evers MB, et al. (2012) FAK is a critical regulator of neuroblastoma liver metastasis. *Oncotarget* 3: 1576-1587.
 57. Polishchuk AL, Li R, Hill-Kayser C, Little A, Hawkins RA, et al (2014) Likelihood of bone recurrence in prior sites of metastasis in patients with high-risk neuroblastoma. *Int J Radiat Oncol Biol Phys* 89: 839-845.
 58. Salazar BM, Balczewski EA, Ung CY, Zhu S (2016) Neuroblastoma, a Paradigm for Big Data Science in Pediatric Oncology. *International journal of molecular sciences* 18: 1.
 59. Harvey H, Piskareva O, Creevey L, Alcock LC, Buckley PG, et al. (2015) Modulation of chemotherapeutic drug resistance in neuroblastoma SK-N-AS cells by the neural apoptosis inhibitory protein and miR-520f. *Int J Cancer* 136: 1579-1588.
 60. Chrystal U Louis JMS (2015) Neuroblastoma: Molecular Pathogenesis and Therapy. *Annu Rev Med* 66: 49-63.
 61. Louis CU, Shohet JM (2015) Neuroblastoma: molecular pathogenesis and therapy. *Annu Rev Med* 66: 49-63.
 62. Betters E, Liu Y, Kjaeldgaard A, Sundstrom E, Garcia-Castro MI (2010) Analysis of early human neural crest development. *Dev Biol* 344: 578-592.
 63. Adamson PC, Houghton PJ, Perilongo G, Pritchard-Jones K (2014) Drug discovery in paediatric oncology: roadblocks to progress. *Nat Rev Clin Oncol* 11: 732-739

64. Bosse KR, Maris JM (2016) Advances in the translational genomics of neuroblastoma: From improving risk stratification and revealing novel biology to identifying actionable genomic alterations. *Cancer* 122: 20-33.
65. Beck B, Blanpain C (2013) Unravelling cancer stem cell potential. *Nature reviews Cancer* 13: 727-738.
66. Ngan ES (2015) Heterogeneity of neuroblastoma. *Oncoscience* 22: 837-838.
67. Rinon A, Molchadsky A, Nathan E, Yovel G, Rotter V, et al. (2011) p53 coordinates cranial neural crest cell growth and epithelial-mesenchymal transition/delamination processes. *Development* 138: 1827-1838.
68. Kim E, Shohet J (2009) Targeted molecular therapy for neuroblastoma: the ARF/MDM2/p53 axis. *J Natl Cancer Inst* 101: 1527-529.
69. Whittle SB, Smith V, Doherty E, Zhao S, McCarty S, et al. (2017) Overview and recent advances in the treatment of neuroblastoma. *Expert Rev Anticancer Ther* 17: 369-386.
70. London WB, Castleberry RP, Matthay KK, Look AT, Seeger RC, et al. (2005) Evidence for an age cutoff greater than 365 days for neuroblastoma risk group stratification in the Children's Oncology Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 23: 6459-6465.
71. Latorre V, Diskin SJ, Diamond MA, Zhang H, Hakonarson H, et al. (2012) Replication of neuroblastoma SNP association at the BARD1 locus in African-Americans. *Cancer Epidemiol Biomarkers Prev* 21: 658-663.
72. Moreno L, Marshall LV, Pearson AD (2013) At the frontier of progress for paediatric oncology: the neuroblastoma paradigm. *Br Med Bull* 108: 173-188.
73. Park JR, Bagatell R, London WB, Maris JM, Cohn SL, et al. (2013) Children's Oncology Group's 2013 blueprint for research: neuroblastoma. *Pediatr Blood Cancer* 60: 985-993.
74. Bagatell R, Beck-Popovic M, London WB, Zhang Y, Pearson AD, et al. (2009) Significance of MYCN amplification in international neuroblastoma staging system stage 1 and 2 neuroblastoma: a report from the International Neuroblastoma Risk Group database. *J Clin Oncol* 27: 365-370.
75. Huang M, Weiss WA (2013) Neuroblastoma and MYCN. *Cold Spring Harb Perspect Med*.
76. Maris JM (2010) Recent advances in neuroblastoma. *N Engl J Med* 362: 2202-2211.
77. Di Giannatale A, Dias-Gastellier N, Devos A, Mc Hugh K, Boubaker A, et al. (2014) Phase II study of temozolomide in combination with topotecan (TOTEM) in relapsed or refractory neuroblastoma: a European Innovative Therapies for Children with Cancer-SIOP-European Neuroblastoma study. *European journal of cancer* 50: 170-177.
78. Schulte JH, Lindner S, Bohrer A, Maurer J, De Preter K, et al. (2013) MYCN and ALKF1174L are sufficient to drive neuroblastoma development from neural crest progenitor cells. *Oncogene* 32: 1059-1065.
79. Hara J (2012) Development of treatment strategies for advanced neuroblastoma. *Int J Clin Oncol* 17: 196-203.
80. Das S, Bryan K, Buckley PG, Piskareva O, Bray IM, et al. (2013) Modulation of neuroblastoma disease pathogenesis by an extensive network of epigenetically regulated microRNAs. *Oncogene* 32: 2927-2936.
81. Pandey GK, Mitra S, Subhash S, Hertwig F, Kanduri M, et al. (2014) The risk-associated long noncoding RNA NBAT-1 controls neuroblastoma progression by regulating cell proliferation and neuronal differentiation. *Cancer cell* 26: 722-737.
82. Zhang Y, Huang D, Zhang W, Tang S, Han T, et al. (2016) Clinical characteristics of infant neuroblastoma and a summary of treatment outcome. *Oncol Lett* 12: 5356-5362.
83. Blumrich A, Zapatka M, Brueckner LM, Zheglo D, Schwab M, et al. (2011) The FRA2C common fragile site maps to the borders of MYCN amplicons in neuroblastoma and is associated with gross chromosomal rearrangements in different cancers. *Hum Mol Genet* 20: 1488-1501.
84. Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, et al. (2009) The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. *J Clin Oncol* 27: 289-297.
85. Michels E, Vandesompele J, De Preter K, Hoebeeck J, Vermeulen J, et al. (2007) ArrayCGH-based classification of neuroblastoma into genomic subgroups. *Genes Chromosomes Cancer* 46: 1098-1108.
86. Strother DR, London WB, Schmidt ML, Brodeur GM, Shimada H, et al. (2012) Outcome after surgery alone or with restricted use of chemotherapy for patients with low-risk neuroblastoma: results of Children's Oncology Group study P9641. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 30: 1842-1848.
87. Baker DL, Schmidt ML, Cohn SL, Maris JM, London WB, et al. (2010) Outcome after reduced chemotherapy for intermediate-risk neuroblastoma. *N Engl J Med* 363: 1313-1323.
88. Ozkaynak MF, Gilman AL, London WB, Naranjo A, Diccianni MB, et al. (2018) A Comprehensive Safety Trial of Chimeric Antibody 14.18 With GM-CSF, IL-2, and Isotretinoin in High-Risk Neuroblastoma Patients Following Myeloablative Therapy: Children's Oncology Group Study ANBL0931. *Frontiers in Immunology* 9: 1-14.
89. Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, et al. (2010) Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *N Engl J Med* 363: 1324-1334.
90. Kushner BH, LaQuaglia MP, Modak S, Wolden SL, Basu EM, et al. (2017) MYCN-amplified stage 2/3 neuroblastoma: excellent survival in the era of anti-GD2 immunotherapy. *Oncotarget*.
91. Guan J, Fransson S, Siaw JTT, Treis D, Van den Eynden J, et al. (2018) Clinical response of the novel activating ALK-I1171T mutation in neuroblastoma to the ALK inhibitor ceritinib. *Cold Spring Harb Mol Case Stud*.
92. Li T, LoRusso P, Maitland ML, Ou SH, Bahceci E, et al. (2016) First-in-human, open-label dose-escalation and dose-expansion study of the safety, pharmacokinetics, and antitumor effects of an oral ALK

- inhibitor ASP3026 in patients with advanced solid tumors. *J Hematol Oncol* 9: 23.
93. Guan J, Tucker ER, Wan H, Chand D, Danielson LS, et al. (2016) The ALK inhibitor PF-06463922 is effective as a single agent in neuroblastoma driven by expression of ALK and MYCN. *Dis Model Mech* 9: 941-952.
 94. Islam SS, Mokhtari RB, Akbari P, Hatina J, Yeger H, et al. (2016) Simultaneous Targeting of Bladder Tumor Growth, Survival, and Epithelial-to-Mesenchymal Transition with a Novel Therapeutic Combination of Acetazolamide (AZ) and Sulforaphane (SFN). *Target Oncol* 11: 209-227.
 95. Hauschild A, Trefzer U, Garbe C, Kaehler KC, Ugurel S, et al. (2008) Multicenter phase II trial of the histone deacetylase inhibitor pyridylmethyl-N-[4-[(2-aminophenyl)-carbamoyl]-benzyl]-carbamate in pretreated metastatic melanoma. *Melanoma Res* 18: 274-278.
 96. Pan LN, Lu J, Huang B (2007) HDAC inhibitors: a potential new category of anti-tumor agents. *Cell Mol Immunol* 4: 337-343.
 97. Singh A, Patel P, Jageshwar, Patel VK, Jain DK, et al. (2017) The Safety, Efficacy and Therapeutic Potential of Histone Deacetylase Inhibitors with Special Reference to Panobinostat in Gastrointestinal Tumors: A Review of Preclinical and Clinical Studies. *Curr Cancer Drug Targets*.
 98. Mottamal M, Zheng S, Huang TL, Wang G (2015) Histone deacetylase inhibitors in clinical studies as templates for new anticancer agents. *Molecules* 20: 3898-3941.
 99. Rettig I, Koenke E, Trippel F, Mueller WC, Burhenne J, et al. (2015) Selective inhibition of HDAC8 decreases neuroblastoma growth in vitro and in vivo and enhances retinoic acid-mediated differentiation. *Cell Death Dis* 6: 1657.
 100. Monti SM, Supuran CT, De Simone G (2013) Anticancer carbonic anhydrase inhibitors: a patent review (2008 - 2013). *Expert Opin Ther Pat* 23: 737-749.
 101. Bayat Mokhtari R, Baluch N, Ka Hon Tsui M, Kumar S, T SH, et al. (2017) Acetazolamide potentiates the anti-tumor potential of HDACi, MS-275, in neuroblastoma. *BMC Cancer* 17: 156.
 102. Kumar S, Mokhtari RB, Sheikh R, Wu B, Zhang L, et al. (2011) Metronomic oral topotecan with pazopanib is an active antiangiogenic regimen in mouse models of aggressive pediatric solid tumor. *Clinical cancer research: an official journal of the American Association for Cancer Research* 17: 5656-5667.
 103. Traore F, Togo B, Pasquier E, Dembele A, Andre N (2013) Preliminary evaluation of children treated with metronomic chemotherapy and valproic acid in a low-income country: Metro-Mali-02. *Indian J Cancer* 50: 250-253.
 104. Fousseyni T, Diawara M, Pasquier E, Andre N (2011) Children treated with metronomic chemotherapy in a low-income country: METRO-MALI-01. *J Pediatr Hematol Oncol* 33: 31-34.
 105. Zhang L, Marrano P, Kumar S, Leadley M, Elias E, et al. (2013) Nab-paclitaxel is an active drug in preclinical model of pediatric solid tumors. *Clinical cancer research : an official journal of the American Association for Cancer Research* 19: 5972-5983.
 106. Moreno L, Casanova M, Chisholm JC, Berlanga P, Chastagner PB, et al. (2018) Phase I results of a phase I/II study of weekly nab-paclitaxel in paediatric patients with recurrent/refractory solid tumours: A collaboration with innovative therapies for children with cancer. *European journal of cancer* 100: 27-34.
 107. Canizares MA, Matilla JM, Cueto A, Algar J, Muguruza I, et al. (2014) Atypical carcinoid tumours of the lung: prognostic factors and patterns of recurrence. *Thorax* 69: 648-653.
 108. Cao C, Yan TD, Kennedy C, Hendel N, Bannon PG, et al. (2011) Bronchopulmonary carcinoid tumors: long-term outcomes after resection. *Ann Thorac Surg* 91: 339-343.
 109. Brodeur GM, Bagatell R (2014) Mechanisms of neuroblastoma regression. *Nat Rev Clin Oncol* 11: 704-713.
 110. Gaze MN, Boterberg T, Dieckmann K, Hormann M, Gains JE, et al. (2013) Results of a quality assurance review of external beam radiation therapy in the International Society of Paediatric Oncology (Europe) Neuroblastoma Group's High-risk Neuroblastoma Trial: a SIOPEN study. *Int J Radiat Oncol Biol Phys* 85: 170-174.
 111. Kaifi JT, Kayser G, Ruf J, Passlick B (2015) The Diagnosis and Treatment of Bronchopulmonary Carcinoid. *Dtsch Arztebl Int* 112: 479-485.
 112. Garner EF, Beierle EA (2015) Cancer Stem Cells and Their Interaction with the Tumor Microenvironment in Neuroblastoma. *Cancers (Basel)* 8: 1.
 113. Pandian V, Ramraj S, Khan FH, Azim T, Aravindan N (2015) Metastatic neuroblastoma cancer stem cells exhibit flexible plasticity and adaptive stemness signaling. *Stem Cell Res Ther* 6: 2.
 114. Bayat Mokhtari R, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, et al. (2017) Combination therapy in combating cancer. *Oncotarget* 8: 38022-38043.
 115. Falasca M, Linton KJ (2012) Investigational ABC transporter inhibitors. *Expert Opin Investig Drugs* 21: 657-666.
 116. Leung EL, Fiscus RR, Tung JW, Tin VP, Cheng LC, et al. (2015) Non-small cell lung cancer cells expressing CD44 are enriched for stem cell-like properties. *PloS one*.
 117. Zhang WC, Shyh-Chang N, Yang H, Rai A, Umashankar S, et al. Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. *Cell* 148: 259-272.
 118. Tang NN, Zhu H, Zhang HJ, Zhang WF, Jin HL, et al. (2014) HIF-1 α induces VE-cadherin expression and modulates vasculogenic mimicry in esophageal carcinoma cells. *World journal of gastroenterology* 20: 17894-17904