

REVIEW

Preclinical drug studies in MEN1-related neuroendocrine neoplasms (MEN1-NENs)

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Abstract

Neuroendocrine neoplasms (NENs) occur usually as sporadic tumours; however, rarely, they may arise in the context of a hereditary syndrome, such as multiple endocrine neoplasia type 1 (MEN1), an autosomal dominant disorder characterised by the combined development of pancreatic NENs (pNENs) together with parathyroid and anterior pituitary tumours. The therapeutic decision for sporadic pNENs patients is multi-disciplinary and complex: based on the grade and stage of the tumor, various options (and their combinations) are considered, such as surgical excision (either curative or for debulking aims), biological drugs (somatostatin analogues), targeted therapies (mTOR inhibitors or tyrosine kinases (TK)/receptors inhibitors), peptide receptor radioligand therapy (PRRT), chemotherapy, and liver-directed therapies. However, treatment of MEN1-related NENs' patients is even more challenging, as these tumours are usually multifocal with co-existing foci of heterogeneous biology and malignant potential, rendering them more resistant to the conventional therapies used in their sporadic counterparts, and therefore associated with a poorer prognosis. Moreover, clinical data using standard therapeutic options in MEN1-related NENs are scarce. Recent preclinical studies have identified potentially new targeted therapeutic options for treating MEN1-associated NENs, such as epigenetic modulators, Wnt pathway-targeting β -catenin antagonists, Ras signalling modulators, Akt/mTOR signalling modulators, novel somatostatin receptors analogues, anti-angiogenic drugs, as well as MEN1 gene replacement therapy. The present review aims to summarize these novel therapeutic opportunities for NENs developing in the context of MEN1 syndrome, with an emphasis on pancreatic NENs, as they are the most frequent ones studied in MEN1-NENs models to date; moreover, due to the recent shifting nomenclature of 'pituitary adenomas' to 'pituitary neuroendocrine neoplasms', relevant data on MEN1-pituitary tumours, when appropriate, are briefly described.

Key Words

- ▶ preclinical studies
- ▶ endocrine neoplasia
- ▶ MEN1
- ▶ neuroendocrine neoplasms

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Introduction

Multiple endocrine neoplasia (MEN) syndromes

The term multiple endocrine neoplasia (MEN) refers to hereditary neoplastic disorders involving two or more endocrine glands within a single patient. Based on specific mutations inducing the development of specific tumours within specific endocrine glands, four major subtypes of MEN are recognized and referred to as types 1–4 (MEN1 to MEN4) (Thakker 2014). The most frequent among these conditions is MEN1, discussed below. MEN2 and MEN3 are induced by mutations in the *RET* (rearranged in transfection) proto-oncogene; MEN2 is characterized by the co-appearance of medullary thyroid carcinoma, pheochromocytoma and parathyroid tumours, whereas MEN3 manifests with MEN2 features except for parathyroid involvement, in the presence of a marfanoid habitus and ganglioneuromas of the lips, tongue and colon (Brandi *et al.* 2001). MEN4 is caused by germline mutations in *CDKN1B* tumor suppressor gene, commonly presenting with parathyroid and pituitary neoplasias (Alrezk *et al.* 2017). As MEN4 is sometimes

confused for MEN1, and as rarely there is an overlap between MEN2 occurring tumours and MEN1 (Naziat *et al.* 2013), we briefly summarize the MEN syndromes-related neuroendocrine neoplasms (NENS) in Table 1.

Multiple endocrine neoplasia type 1 (MEN1, Wermer's syndrome)

MEN1 was firstly described in the early twentieth century; however, it was not until 1954 when Wermer described familial occurrence in which a father and four of the nine offspring were affected (Wermer 1954). Patients with MEN1 characteristically develop tumours of the parathyroid glands with primary hyperparathyroidism (~95%), the anterior pituitary (~30%), and the pancreatic islets (~40%); less commonly, adrenal cortical adenomas/carcinoma, thyroid follicular adenomas, extra-pancreatic neuroendocrine neoplasms such as duodenal gastrinoma or gastric, thymic, and bronchial carcinoids may appear (Duh *et al.* 1987, Grama *et al.* 1992, Skogseid *et al.* 1992, Trump *et al.* 1996, Thakker 2000, Thakker *et al.* 2012).

MEN1 is an autosomal dominant disorder induced by mutations in the tumor suppressor gene *MEN1*,

Table 1 MEN syndromes and related neuroendocrine neoplasms.

MEN syndromes-related NENS (chromosome location, gene, function)	Site (estimated penetrance)	Specific NEN
MEN1 (11q13.1; <i>MEN1</i> ; tumour suppressor gene) (Thakker <i>et al.</i> 2012)	Enteropancreatic (30–70%) ‘Foregut’ (2–10%) Pituitary (30–40%) PPGL (extremely rare)	Gastrinomas (>40%) Insulinomas (10–30%) Glucagonomas (~3%) VIPomas (<1%) NF pNENS (20–55%) Somatostatinomas (extremely rare) Other (e.g., GHRH-/PTH-RP-/ACTH-secreting) Thymic, bronchial, gastric NENS Prolactinomas (20%) ACTH, TSH, GH+PRL, GH, NF (each 2–9%) Pheochromocytomas (<1%)
MEN2 (MEN2A) (10q11.2; <i>RET</i> ; proto-oncogene) (Brandi <i>et al.</i> 2001)	Thyroid (90%) PPGL (50%) Pituitary ^a	MTC (90%) Pheochromocytomas (50%) ACTH-secreting (Naziat <i>et al.</i> 2013)
MEN3 (MEN2B) (10q11.2; <i>RET</i> ; proto-oncogene) (Brandi <i>et al.</i> 2001, Thakker 2014)	Thyroid (>90%) PPGL (40–50%) Pituitary ^a	MTC (>90%) Pheochromocytomas (40–50%) ACTH-secreting (single case report) (Kasturi <i>et al.</i> 2017)
MEN4 (12p13.1; <i>CDKN1B</i> ; tumour suppressor gene) (Thakker 2014, Alrezk <i>et al.</i> 2017)	Enteropancreatic ^a ‘Foregut’ ^a Pituitary ^a Other ^a	Duodenal and pancreatic NENS (Agarwal <i>et al.</i> 2009, Tonelli <i>et al.</i> 2014) Bronchial and gastric NENS (Molatore <i>et al.</i> 2010, Malanga <i>et al.</i> 2012) ACTH-/GH-secreting, NF (Pellegata <i>et al.</i> 2006, Tichomirowa <i>et al.</i> 2012, Occhi <i>et al.</i> 2013, Sambugaro <i>et al.</i> 2015) Small-cell neuroendocrine cervical carcinoma (Georgitsi 2010)

^aInsufficient numbers reported to provide prevalence information. ACTH, adrenocortical hormone; CDKN1B, cyclin-dependent kinase inhibitor 1B (p27Kip1) gene; GHRH, growth hormone releasing hormone; MTC, medullary thyroid carcinoma; NENS, neuroendocrine neoplasms; NF, non-functioning; pNENS, pancreatic neuroendocrine neoplasms; PRL, prolactin; RET, rearranged during transfection gene; TSH, thyroid stimulating hormone; VIPomas, vasointestinal polypeptide secreting tumours.

which encodes a 610-amino acid protein, menin (Thakker *et al.* 2012, Kamlaris & Stratakis 2019). However, in up to 10–30% of MEN1 patients no mutation in the *MEN1* gene can be diagnosed, as the regular approaches fail to detect possible mutations in non-coding and regulatory regions as well as to identify phenocopies. Next-generation sequencing (NGS), a novel sequencing technology, may bypass these limitations, increasing the strength and efficacy of genetic analysis (Marini *et al.* 2015, de Laat *et al.* 2016).

Prior to 1980, ~80% of MEN1-related deaths were caused by gastrinoma-derived gastric acid hypersecretion inducing multiple gastro-intestinal ulcers, bleeding and perforation; improvements in pharmacological control of the hypergastrinemia and related gastric acid hypersecretion have strongly reduced mortality related to these complications. Noteworthy, most of MEN1-related pancreatic or thymic NENs patients (~70–90%) will require therapeutic intervention during their life with the disease, including surgery and/or systemic therapy (somatostatin analogues, peptide-receptor radioligand therapy, everolimus, loco-regional therapies or chemotherapy) due to tumor progression/recurrence/multi-focality (De Laat *et al.* 2014, Faggiano *et al.* 2020, Oleinikov *et al.* 2020). Despite the advances in their treatment, the life expectancy of MEN1-patients remains shorter than normal population (mean age at death ~55 years) (Norton *et al.* 2015), with death prevalently occurring as result of the malignant progression of pancreatic and thymic NENs, which are responsible for ~50% and ~24% of fatalities, respectively (Marini *et al.* 2017).

MEN1-NENs: limitations of current therapies

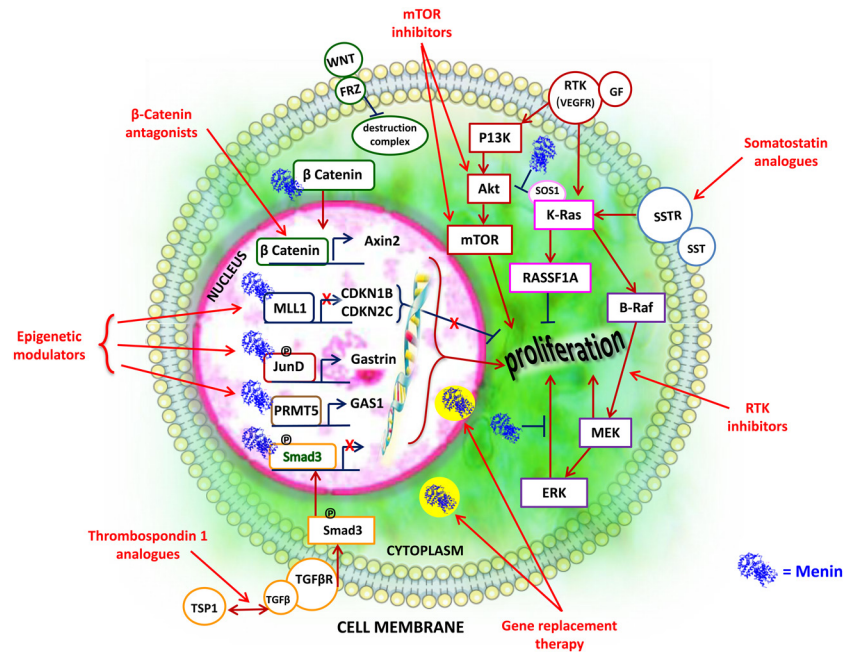
The existing therapeutic options for the various NENs have not been formally evaluated in MEN1 patients, being extrapolated from non-MEN1 NENs patients (Pieterman *et al.* 2020). There is scarcity of evidence reporting on these anti-tumour therapies specifically in MEN1-NENs patients. The optimal treatment for these patients is challenging, as MEN1-NENs are multiple, multicentric, pose a higher metastatic potential and are relatively insensitive to treatment (Dean *et al.* 2000, Frost *et al.* 2018). The multi-focality of MEN1-NENs and their unpredictable malignant potential pose difficulties for the timing and extent of curative surgery. As a result, these patients frequently require additional non-surgical treatments, such as biotherapies (somatostatin analogues, SSAs), molecular-targeted therapies (mTOR

inhibitors or tyrosine kinase (TK)/receptors inhibitors), peptide-receptor radioligand therapy (PRRT), chemotherapy and/or loco-regional therapies. The choice of optimal anti-tumour therapies for MEN1-NENs patients is challenging and needs the involvement of experienced multi-disciplinary teams inside referral centers of excellence.

Preclinical studies on emerging therapies in MEN1-NENs

Since the discovery of the *MEN1* gene in 1997, the elucidation of the molecular function of its protein product, menin, has been challenging; nonetheless, biochemical, proteomics, genetics and genomics approaches have identified many potential roles for menin, which all converge on gene expression regulation (Dreijerink *et al.* 2017). Briefly, menin is ubiquitously expressed and functions as a nuclear key scaffold protein in a tissue-specific manner, displaying opposing roles between different organs, either as a bona fide tumor *suppressor* in endocrine organs yet essential as *promoter* of leukemogenesis in mouse models; these effects are probably the result of menin's capacity to dichotomously regulate gene expression, as well as to functionally crosstalk with a multitude of proteins and signalling pathways involved in cell behaviour such as gene transcription, genome stability, cell division, cell cycle control and epigenetic regulation (Matkar *et al.* 2013). Specifically, in the nucleus, menin interacts with the transcription factor JunD and the protein arginine methyltransferase (PRMT) 5 to suppress transcription of target genes; it binds to chromatin-modifying protein complexes such as the histone modifiers mixed lineage leukemia proteins (MLL)-1 and -2-containing complexes and Smad3 (a TGF- β signalling component) to promote transcription of target genes; it restricts Wnt pathway target genes transcription by blocking β -catenin from entering the nucleus; in the cytoplasm, menin binds to Akt inhibiting the mechanistic target of rapamycin (mTOR pathway) downstream of PI3K and hampers ERK-dependent K-Ras phosphorylation preventing the interaction between the guanine nucleotide exchange factor son of sevenless (SOS) and K-Ras (Stalberg *et al.* 2004, Milne *et al.* 2005, Wang *et al.* 2011, Chamberlain *et al.* 2014, Frost *et al.* 2018) (Fig. 1).

Preclinical in vitro and in vivo models of cancer are instrumental in studying genes functions, in deciphering the biology of tumor initiation and progression, and in performing preclinical studies aimed at testing

**Figure 1**

Menin-associated tumourigenic pathways and the possible targeting therapies to be considered. Menin, which is encoded by the *MEN1* gene, has several nuclear and cytoplasmic functions. Loss of expression of the tumour suppressor protein menin (drawn as blue-azure symbol) results in increased cell proliferation by altering multiple different signalling pathways. In the nucleus, menin can alter gene transcription by: interacting with JunD or the protein arginine N-methyltransferase (PRMT) 5 to repress transcription of target genes, e.g. *Gastrin* and *Gas1*, respectively; binding to mixed lineage leukaemia proteins MLL1 and/or MLL2, and the TGF- β signalling component Smad3, to promote transcription of target genes; and regulates the Wnt pathway by preventing β -catenin from entering the nucleus and therefore preventing transcription of Wnt pathway target genes. In the cytoplasm, menin inhibits: the mTOR pathway by binding to Akt, which is downstream of PI3K in the receptor tyrosine kinase (RTK) signalling pathway, and preventing its translocation to the plasma membrane; and K-Ras induced proliferation, by possible inhibition of ERK dependent phosphorylation and prevention of the interaction between SOS and K-Ras. GF, growth factor; P13K, phosphoinositide 3-kinase; Akt, protein kinase B; mTOR, mechanistic target of rapamycin; FRZ, fizzled; MLL, mixed lineage leukemia; CDKN, cyclin dependent kinase inhibitor; GAS1, growth arrest specific 1; SMAD3, mothers against decapentaplegic hormone 3; TGF β (R), transforming growth factor beta (receptor); TSP1, thrombospondin 1; SST(R), somatostatin (receptor); SOS1, sons of sevenless 1; RASSF1A, ras associated domain family member 1 isoform A; MEK, mitogen activated protein kinase; ERK, extra signal-related kinase. Data from [Frost *et al.* \(2018\)](#).

novel therapies. Several MEN1 animal models have been generated in different organisms by introducing loss-of-function mutations in the orthologues of the human MEN1 gene, which closely resemble the tumor spectrum and associated hormonal changes seen in human disease, although individual tumor behaviour may be variable ([Wiedemann & Pellegata 2016](#), [Mohr & Pellegata 2017](#)). The increased understanding of menin function has allowed for the preclinical development of menin-targeted therapies for NENs, which are discussed below. Noteworthy, most of the research performed to date on MEN1-NENs almost exclusively involves pancreatic tumours extracted from MEN1-mice models, which will be therefore elaborated in the present review; moreover, due to the recent shifting nomenclature of 'pituitary adenomas' to 'pituitary neuroendocrine neoplasms', relevant data on MEN1-pituitary tumours, when appropriate, are briefly described ([Asa *et al.* 2017](#)).

Epigenetic modulators

Menin has been demonstrated to play a role in gene transcription, through the regulation of epigenetic mechanisms, including histone modifications. For example, menin has been shown to interact with a number of histone-modifying proteins including histone methyltransferases (e.g. mixed lineage leukaemia 1 (MLL1) and protein arginine methyltransferase 5 (PRMT5)) and acetyltransferase complexes (HDACs) to regulate the expression of tumor suppressor genes including *CDKN1B* and *GAS1* ([Pieterman *et al.* 2014](#), [Thakker 2014](#)). The use of epigenetic-targeting compounds may therefore have utility in MEN1-associated tumours. Preclinical studies have indicated that JQ1, an inhibitor of the bromo and extra terminal domain (BET) family of proteins that bind to acetylated histone residues to promote gene transcription may have efficacy in pancreatic, bronchial and pituitary NENs as in vitro studies revealed that JQ1 decreased proliferation and increased apoptosis of

pancreatic, pituitary and bronchial NEN cell lines, as well as reducing ACTH secretion from the ACTH-secreting pituitary cell lines, AtT20 (Lines *et al.* 2017, 2020). Furthermore, in vivo, assessment using a pancreatic β -cell-specific conditional Men1-knockout mouse model that develops pNENs, revealed that JQ1 decreased proliferation and increased apoptosis of pNENs (Lines *et al.* 2017). In addition, CPI203, another BET inhibitor, has also been reported to reduce pNEN proliferation in a BON-1 xenograft model (Wong *et al.* 2014).

To date, the potential utility of histone deacetylases inhibitors (HDACi) has been demonstrated in some sporadic NENs, whereas specific studies on MEN1-NENs models are limited. Namely, inhibition of HDAC5 using the compound LMK-235 has been reported as a potential therapeutic target in pNENs (Wanek *et al.* 2018). In addition, the class I HDAC1/3 inhibitor etinostat was able to inhibit master regulator proteins in 42% of metastatic gastro-entero-pancreatic NENs, and to reduce tumour growth in a small intestinal NEN xenograft mouse model (Alvarez *et al.* 2018). The HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) has also been reported to decrease proliferation and increase apoptosis, this time in a GH3 rat pituitary NEN cell line (Sangeetha *et al.* 2009). Recently, some HDACi such as vorinostat (SAHA), romidepsin or panobinostat were approved by FDA for specific hematologic malignancies (Suraweera *et al.* 2018); however, limited efficacy has been shown in patients with sporadic pNENs (Jin *et al.* 2016). Although epigenetic modulators seem attractive as potential therapies for NENs patients, further clinical studies using selective compounds alone or in combination with other anticancer agents are needed to understand their real therapeutic potential, and specifically for MEN1-NENs patients.

In addition, it is well established that menin forms a complex with MLL and Ash2 proteins to promote histone 3, lysine 4 (H3K4) methylation, which in turn increases the expression of anti-proliferative genes including cyclin-dependent kinase (CDK) inhibitors that encode p27 and p18 (Hughes *et al.* 2004, Karnik *et al.* 2005, Milne *et al.* 2005). Furthermore, preclinical studies have demonstrated that genetic ablation of the retinoblastoma binding protein 2 (Rbp2), which acts as a H3K4 demethylase, can reduce proliferation of pNEN cells, and reduce pancreatic tumour burden in a *Men1* conditional knockout mice (Lin *et al.* 2011, Maggi *et al.* 2016). Moreover, Rbp2 expression has been reported to be elevated in NENs (Maggi *et al.* 2016). Therefore, modulators of CDK expression, including inhibitors of

Rbp2 could provide a novel therapeutic approach for MEN1-associated NENs.

Wnt pathway-targeting β -catenin antagonists

Menin can reduce cell proliferation through Wnt/ β -catenin signalling by promoting β -catenin phosphorylation and its transfer from the nucleus. The absence of menin leads to nuclear β -catenin accumulation and transcriptional activation of the target genes (Matkar *et al.* 2013). As previously mentioned, menin possesses dichotomous functions by positively or negatively regulating different gene expression and by interacting with a multitude of proteins with diverse functions. It was suggested that menin may either promote or inhibit Wnt signalling in certain stages of islet tumor development as well as in certain cell types: for example, menin was shown to be essential for canonical Wnt/ β -catenin signalling in cultured rodent islet tumor cells yet it was suggested that it may also inhibit Wnt signalling to prevent β cells from early-stage tumorigenesis; however, in Men1-null mouse embryonic fibroblasts (MEF) menin promoted nuclear export of β -catenin, suppressing its transcriptional activity. Nevertheless, the menin/Wnt/ β -catenin interactions remain yet to be explored.

Data on Wnt signalling modulators in MEN1-related tumor models is limited. In a study using both Men1-null mouse embryonic fibroblasts (MEF) and insulinoma tissues from β -cell-specific Men1-knockout mice (with a nuclear accumulation of β -catenin), Cao *et al.* have shown that overexpression of menin reduces β -catenin nuclear accumulation and its transcriptional activity, and that menin directly interacts with β -catenin carrying it out of the nucleus via nuclear-cytoplasmic shuttling (Cao *et al.* 2009).

In another study using MEN1-deficient mice developing pNENs (RIP-Cre, with pancreatic β -cell conditional knockout of menin), the additional conditional knockout of β -catenin decreased the number and the size of pancreatic tumours and increased mice survival (Jiang *et al.* 2014). Moreover, the use of a β -catenin antagonist (PKF115-584) decreased pNEN cell proliferation, suggesting that Wnt-signalling modulators may provide a novel approach for the treatment of MEN1-NENs patients.

Ras/Raf/MEK/ERK pathway modulators

Aberrant activation of the RAS-RAF-MEK-ERK (MAPK) pathway is implicated in numerous cancers. Initial studies have been shown that NENs display

activating mutations in the rat sarcoma (Ras) family of signal-transducing genes, over-activity of p21(Ras)-signalling pathways, or constitutive activation of upstream or downstream effectors of Ras including growth factor receptors or PI(3)-kinase and Raf/mitogen-activated protein kinases; Ras depends on protein kinase C delta (PKC δ)-mediated survival pathways (Xia *et al.* 2007). In a study by Kim *et al.*, menin overexpression in a pro-oncogenic RAS-transformed murine NIH3T3 cells decreased cell proliferation and tumor growth in athymic mice, restraining RAS oncogenic effects (Kim *et al.* 1999).

K-Ras, a member of the Ras family, paradoxically suppressed growth in pancreatic endocrine cells in a mice model, and this effect depended on the antiproliferative Ras effector RASSF1A and blockade of the Raf/MAPK pathway by menin; stimulation of ERK1/2 phosphorylation combined with a menin inhibitor synergistically enhanced proliferation, whereas inhibition of MAPK signalling created a lethal effect in the setting of menin loss. These insights suggest potential strategies for targeting menin-sensitive endocrine tumors (Chamberlain *et al.* 2014). Recently, using an ATRX-specific Kras^{G12D/+}/Men1^{-/-} driven genetically engineered mouse model, it was shown that deficiency of menin results in the accumulation of DNA damage and antagonizes oncogenic Kras-induced senescence during tumorigenesis (Qiu *et al.* 2020).

Following promising pre-clinical data, the novel selective ERK1/2 inhibitor BVD-523 (ulixertinib) entered clinical trials with encouraging antitumor activity in patients with solid tumours harbouring mutations in the MAPK/ERK pathway; however, data on NENs and specifically on MEN-NENs, is still limited (Germann *et al.* 2017, Sullivan *et al.* 2018).

Akt/mTOR signalling modulators

Mammalian (mechanistic) target of rapamycin (mTOR), a serine/threonine protein kinase involved in the regulation of different cellular functions (cell proliferation, migration, activation of transcription factors, etc.), is constitutively activated in NENs as a catalytic subunit of 2 distinct complexes: the rapamycin-sensitive mTORC1 which activates the downstream protein-kinase S6k and eIF4B, inducing cell proliferation; and the rapamycin-insensitive mTORC2, which compensatory phosphorylates Akt promoting its overactivation and the development of resistance to mTORC1 inhibitors (mTORi) such as rapamycin, respectively (Grozinsky-Glasberg & Pavel 2012).

Although the mTORC1 inhibitor RAD001 (everolimus) is already in clinical use for non-MEN1-NENs of different origins, data on menin and Akt/mTOR pathway signalling is limited. Razmara *et al.* aimed to assess the impact of menin expression (alone, or in addition to co-treatment with rapamycin) on cell proliferation in a cell-line model including menin-silenced BON-1 cells (Razmara *et al.* 2018). Lack of menin enhanced mTORC2-Akt activation as well as the rapamycin-induced pAkt and a direct negative regulation between menin and the rapamycin-mediated mTORC2-Akt activation was observed. Apparently, menin is essential in mTORC1/C2 crosstalk and may influence the response to mTORi in pNENs patients.

Another study by Wang *et al.* also suggested that menin is an important negative regulator of Akt kinase activity, this time using a Men1^{+/-} mice model bearing islet adenomas (Wang *et al.* 2011). IHC staining for pAkt(S473) and menin of 18 months Men1^{+/-} mice derived islet adenomas vs WT demonstrated that expression of pAkt(S473) in islet adenomas correlates with loss of menin expression, meaning that menin downregulates AKT activity and inhibits both Akt induced proliferation and Akt anti-apoptosis effects.

Recently, Wong *et al.* demonstrated that the coexistence of other mutations (e.g. Pten loss) together with Men1 loss accelerate the neuroendocrine tumorigenesis in two genetically engineered mouse models of well differentiated pNENs (Wong *et al.* 2020). Their data highlight the importance of the PI3K/AKT/mTOR pathway in NENs genesis in mice and that treatment with the mTOR inhibitor rapamycin delayed the growth of pNENs as well as the growth of pituitary NENs, resulting in prolonged survival in these mice.

Anti-angiogenic drugs

Angiogenesis, or the formation of new capillary blood vessels, is a fundamental process in cancer development, including in NENs. The proangiogenic signalling molecule vascular endothelial growth factor (VEGF) and its cognate receptor VEGF receptor 2 (VEGFR-2) play a central role in angiogenesis (Zhao & Adjei 2015). Initial antiangiogenic drugs inhibited VEGF/VEGFR signalling inducing a transient response, followed usually by tumor progression as other pathways compensate for the initial inhibition, such as increase in tumour hypoxia, in the expression of pro-angiogenic factors including VEGFA or fibroblast growth factors (FGFs family, ephrin A1, and c-Met activation, etc.); therefore, a simultaneous co-targeting of VEGF, PDGF, FGF, etc. and their receptors may improve clinical outcomes.

Chu *et al.* evaluated the possible structural, molecular and functional microvascular aberrations contributing to development and maintenance of pNENs using a Men1 mouse model (Chu *et al.* 2013). They have showed that the increased vascular density of pNENs in Men1 mice was paralleled by an early and extensive redistribution of pericytes, alterations supported by variations in expression of several angiogenic regulators and potentiated by hypoxia. They also demonstrated that both vascular reactivity/constriction and blood perfusion of tumor arterioles are significantly altered in response to glucose and L-nitro-arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor), suggesting a possible role as therapy.

Few studies evaluated the effect of different anti-angiogenic molecules on tumor growth, invasiveness and metastatic potential using the Rip1Tag2 transgenic mouse model of non-MEN1-pNENs: nintedanib, targeting VEGF, PDGF, FGF receptors and c-Src, induced a strong antiangiogenic response with decrease in tumor growth and no increase in invasiveness or metastatic spread (Bill *et al.* 2015); sunitinib, a VEGF-inhibitor, reduced initially the tumor burden followed by an increase in tumor invasiveness and metastasis, which were reversed eventually by a dual inhibition of c-Met and VEGF signalling with several compounds including crizotinib or cabozantinib (Sennino *et al.* 2012); finally, functionally active peptides derived from endogenous angiogenesis inhibitors (such as tumstatin, endostatin and the second type 1 repeat of thrombospondin-1), suppressed angiogenesis and reduced tumour growth, whereas their genetic-induced deficiency accelerated tumor growth and decreased mice survival (Xie *et al.* 2011). Although these studies were performed in Rip1Tag2 transgenic mouse model, the effects of angiogenesis modulation on tumor control seem promising and further research using MEN1-NENs models is warranted.

Somatostatin analogues (SSAs)

Somatostatin (SST) and its analogues were demonstrated to have anti-proliferative effects in a variety of tumour cells by inhibiting the mitogenic signalling of growth factor receptor kinases, by inducing apoptosis, or by inhibiting the secretion of insulin-like growth factor-I, etc., and are considered today the mainstay of therapy in sporadic NENs (Grozinsky-Glasberg *et al.* 2008). However, data on their effects in MEN1-NENs models is limited.

Quinn *et al.* tested the effect of pasireotide (SOM230, a pan-SST receptor (SSTR) agonist that acts via SSTR1,2,3 and 5), on insulin secretion, glucose levels, tumor growth,

and mice survival using an MEN1 transgenic mouse model (Quinn *et al.* 2012). SOM230 demonstrated significant antisecretory, antiproliferative and proapoptotic activity in the MEN1 model of insulinoma. Moreover, the effects of pasireotide were also evaluated in a Men1^{+/-} mouse model developing pancreatic and pituitary NENs (Walls *et al.* 2016). Pasireotide decreased proliferation and increased apoptosis of pNENs, suppressed tumour growth and tumor number, increased mice survival and resulted in prevention of tumor development. These results suggest the potential utility of SSAs such as pasireotide as chemo-preventive or prophylactic treatment of pancreatic and pituitary NENs in patients with MEN1, as supported by some recent clinical data (Cioppi *et al.* 2017, Faggiano *et al.* 2020). Further prospective studies of the effects of SOM230 in MEN1-NENs patients are warranted. Noteworthy, most of NENs express both SSTR and dopamine receptor 2 (D2DR), and evaluating the potential of SSTR2-D2DR co-targeting in these tumors seem attractive; however, further investigation is required to assess the possible role of chimeric agonists in NENs in general, and particularly in MEN1-NENs (Zitzmann *et al.* 2013, Herrera-Martínez *et al.* 2019).

MEN1 gene replacement ('living drug') studies

The concept of *MEN1* gene replacement therapy is based on the evidence that majority of MEN1-NENs have loss of heterozygosity (LOH) for the MEN1 allele located on chr 11q13, consistent with a tumor suppressor role for menin, and that mutations in menin are associated with tumor development. To date, few studies were performed to investigate whether inducing menin overexpression by viral/ non-viral gene delivery methods could reverse the phenotype in human tumor cells low in menin expression and to suppress tumor cell proliferation.

Walls *et al.* demonstrated initially that Men1^{+/-} mice-originating tumours (pNENs and pituitary NENs) had higher proliferation rates compared with their respective normal tissues (Walls *et al.* 2012a). Using a knockout Men1^{+/-} mouse model which developed pituitary NENs, the transauricular injection of a recombinant adenoviral serotype 5 vector (rAd5) (containing Men1 cDNA, rAd5-MEN1) directly in the pituitary tumours of female mice increased menin expression and decreased tumor proliferation (Walls *et al.* 2012b). Finally, in a pNEN-bearing Men1 gene KO transgenic mice (insulin-secreting) model, the injection of Oct-AAVP-TNF (a hybrid adeno-associated virus and phage (AAVP) vector displaying

active octreotide) decreased tumor metabolism, insulin secretion and tumor size, and improved mice survival (Smith *et al.* 2016). Gene therapy for MEN1-NENs may represent a promising cutting-edge therapy. However, there are major issues to be solved including optimal delivery, risks of several off-target/adverse effects including immune response and mutagenesis and efficacy (Goswami *et al.* 2019). Further pre-clinical research is warranted before defining the possible role of *MEN1* gene replacement therapy in patients with MEN1-NENs.

Conclusions and future directions

Recent studies have further attempted to decipher the complex molecular alterations and pathways involved in the development and progression of MEN1-NENs, including insights in the genes involved in chromatin remodelling and epigenetic regulation, in mTOR-, K-RAS- or b-catenin/Wnt signalling, as well as on SSTR signalling. In addition, preclinical studies have suggested the possible efficacy of epigenetic modulators, Wnt pathway targeting β -catenin antagonists, multi-SSTR-targeted analogues and *MEN1* gene replacement therapy in treating MEN1-related NENs. However, further and throughout evaluation of such emerging treatments in clinical trials is warranted to assess their outcomes and limitations, and before their possible use in patients with MEN1-related NENs. Further research on the tissue-specific actions of menin may reveal potential therapeutic targets and facilitate translational studies in MEN1-NENs. New directions may include the development of specific and advanced MEN1-NENs preclinical models (e.g., novel cellular models, xenograft models, or three-dimensional tumor cell organoid models), evaluating the delay in tumorigenesis after the loss of both copies of the *Men1* gene, as well as further elucidation of the relation between menin loss and the various molecular pathways involved in the tissue-selective anti-tumor function of menin.

Declaration of interest

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