

# **Current and Emerging Therapies for Pancreatic Neuroendocrine Tumours in Patients with or without Multiple Endocrine Neoplasia Type 1**

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## 24    **ABSTRACT**

25    Pancreatic neuroendocrine tumours (PNETs) may occur as a non-familial isolated endocrinopathy  
26    or as part of a complex hereditary syndrome, such as multiple endocrine neoplasia type 1 (MEN1),  
27    which is an autosomal dominant disorder characterised by the combined occurrence of PNETs with  
28    tumours of the parathyroids and anterior pituitary. Treatments for primary PNETs, include surgery,  
29    and for nonresectable PNETs and metastases, treatments include biotherapy (e.g. somatostatin  
30    analogues, inhibitors of receptors, and monoclonal antibodies), chemotherapy, and radionuclide  
31    therapy. However, treatment of PNETs in patients with MEN1 is challenging due to concomitant  
32    development of tumours, which may have metastasised, and there is a scarcity of clinical trials  
33    reporting the effects of these anti-tumour therapies in PNETs of MEN1 patients. For example,  
34    clinical trials have shown that inhibitors of receptor tyrosine kinases (RTKs) and the mechanistic  
35    target of rapamycin receptor (mTOR) pathway, and antibodies to vascular endothelial growth factor  
36    A (VEGFA) are effective treatments for PNETs in non-MEN1 patients, but data from MEN1  
37    patients is lacking. Recent preclinical studies have identified potentially new therapeutic targets for  
38    treating MEN1-associated NETs, and these include epigenetic modification, the  $\beta$ -catenin/Wnt-  
39    pathway, hedgehog signalling, and somatostatin receptors, as well as *MEN1* gene replacement  
40    therapy. This review discusses these advances.

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42

## 43 INTRODUCTION

44

45 Pancreatic neuroendocrine tumours (PNETs) have a reported incidence of 0.48 per 100,000 of the  
46 population, although they are found more frequently in 0.8% to 1.0% of patients undergoing post-  
47 mortem examinations<sup>1-3</sup>. PNETs usually occur as a non-familial (i.e. sporadic) isolated  
48 endocrinopathy, but they may also occur as part of a complex hereditary syndrome, such as multiple  
49 endocrine neoplasia type 1 (MEN1), von-Hippel Lindau disease, von Recklinghausen's syndrome  
50 (Neurofibromatosis type 1, NF1), and tuberous sclerosis<sup>4,5</sup>. PNETs have been reported to occur in  
51 30%-80% of MEN1 patients, >15% of VHL patients, <10% of NFI patients, and <1% patients with  
52 tuberose sclerosis. Thus, MEN1 is the most common hereditary syndrome associated with PNETs,  
53 and ~10% of all PNETs are associated with MEN1<sup>6</sup>. Moreover, somatic mutations of the *MEN1*  
54 gene, which are found in virtually all PNETs of MEN1 patients<sup>7</sup> are also found to occur in >40% of  
55 sporadic PNETs, indicating that *MEN1* mutations are “major drivers” in the development of all  
56 PNETs<sup>8,9</sup>. Current treatment of PNETs, which comprise drugs (e.g. chemotherapy and  
57 biotherapies), surgery, and radiotherapy (Figure 1 and Table 1) are often not successful, such that  
58 the median survival time for patients with PNETs is ~3.6 years<sup>1</sup>. Thus, there is a clinically unmet  
59 need for better treatments, which may arise from a greater understanding of PNET biology and the  
60 role of the *MEN1* gene and its encoded protein menin. This review will focus on providing an  
61 overview of the clinical features (Figure 2) and genetics of MEN1, the functions of menin (Figure  
62 3), the current therapies for PNETs in non-MEN1 patients and their use in treating PNETs in MEN1  
63 patients (Table 1 and Supplementary Table 1), and emerging therapies of which some are based on  
64 the function of menin (Figure 3).

65

## 66 MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 (MEN1)

67 Multiple endocrine neoplasia type 1 (MEN1) is characterised by the combined occurrence of two or  
68 more tumours that usually involve the parathyroids, pancreatic islets, anterior pituitary and adrenals  
69 (Figure 2A-C)<sup>10</sup>. In addition, <5% of MEN1 patients may develop carcinoid tumours of the thymus,  
70 bronchus, and gut, and women may have an increased occurrence of breast cancer<sup>10,11</sup>. The majority  
71 of MEN1-patients will have developed manifestations of an MEN1-tumour by the age of 45 years<sup>12</sup>,  
72 with primary hyperparathyroidism (PHPT), due to parathyroid tumours, being the most common,  
73 and occurring in >80% of MEN1 patients by the age of 50 years<sup>13</sup>. Among the pancreatic islet cell  
74 tumours, also referred to as PNETs, ~60% will secrete polypeptide hormones such as gastrin,  
75 insulin, glucagon, or vasoactive intestinal peptide (VIP), and cause multiple ulcers of the stomach  
76 and duodenum, hypoglycaemia with seizures, glucose intolerance, and watery diarrhoea with  
77 hypokalaemic alkalosis (WDHA) syndrome, respectively<sup>14,15</sup>. However, 40% of these pancreatic  
78 NETs may not secrete hormones, and these are referred to as non-functioning (i.e. non-secreting)  
79 PNETs<sup>10,14,15</sup>. Among the anterior pituitary tumours in MEN1 patients, ~90% will secrete prolactin,  
80 growth hormone (GH) or adrenalcorticotrophic hormone (ACTH), and the remaining ~10% are non-  
81 functioning (or glycoprotein subunit secreting) adenomas<sup>10,15</sup>. Among the adrenal cortical tumours  
82 in MEN1 patients ~10% will hypersecrete glucocorticoids or mineralocorticoids, whilst ~90% will  
83 be non-functioning. Hormonal hypersecretion by these MEN1-associated endocrine tumours, the  
84 majority of which are benign adenomas, will result in hormone related disorders with specific  
85 symptoms and morbidities that are not always similar to those in non-MEN1 patients<sup>10,15</sup>. This is  
86 because non-MEN1 patients will only have a tumour of one endocrine gland, whereas MEN1  
87 patients will have tumours occurring in two or more glands (Figure 2A-D). These endocrine  
88 tumours in MEN1 patients are associated with a decreased 20-year survival of <65%, when  
89 compared to that of >80% in an age and sex-matched US population<sup>16</sup>.

90

## 91    **Genetics of MEN1**

92    MEN1 is an autosomal dominant disorder caused by mutations of the *MEN1* tumour suppressor  
93    gene, which encodes a 610 amino acid protein called menin. *MEN1* germline mutations are found in  
94    >90% of patients<sup>7,10</sup>, and comprise whole or partial gene deletions, frameshift deletions or  
95    insertions, in-frame deletions or insertions, and splice site, missense, and nonsense mutations, and  
96    result in a functional deficiency of menin<sup>10</sup>. There appear to be no genotype-phenotype  
97    correlations<sup>7,17,18</sup>. MEN1 tumours will have somatic mutations as well as the germline mutations,  
98    consistent with the Knudson two-hit hypothesis for the role of tumour suppressor genes in  
99    oncogenesis, and in the majority (>90%) of MEN1 tumours the somatic abnormality is loss of  
100    heterozygosity (LOH), with the remaining 10% having intrageneic deletions or point  
101    mutations<sup>10,16,19,20</sup>. Moreover, the *MEN1* gene is involved in the aetiology of non-MEN1  
102    parathyroid tumours, PNETs, pituitary adenomas, and adrenal cortical adenomas, as ~20%, ~40%,  
103    ~4% and ~2% of these have somatic *MEN1* mutations, respectively<sup>10,21</sup>.

104

## 105    **Current treatment of endocrine tumours in MEN1**

106    The choice of optimal anti-tumour therapies, which comprise medical, surgical, and radiological  
107    approaches (Table 1 and Figure 1), for MEN1 patients is frequently challenging, as such therapies  
108    have not been formally evaluated with clinical trials in MEN1 patients but instead have often been  
109    extrapolated from outcomes of clinical trials reported from non-MEN1 patients who are affected  
110    with a single endocrine tumour (Supplementary Table 1)<sup>21</sup>. This increases reliance on consensus  
111    expert opinions, which acknowledge the uncertainties in the provisions of optimal treatments.  
112    However, all recognise that in the absence of treatment, endocrine tumours in MEN1 patients are  
113    associated with an earlier mortality<sup>21</sup>. Thus, untreated patients with MEN1 tumours have a  
114    decreased life expectancy with a 50% probability of death by the age of 50 years, and the cause of

115 death in 50-70% of patients with MEN1 is usually a malignant tumour process or sequelae of the  
116 disease<sup>19,20</sup>. This increased mortality in MEN1 patients can be attributed to PNETs, which may  
117 metastasise<sup>19,20</sup>. However, the implementation of genetic diagnosis and regular screening for  
118 MEN1-associated tumours for their earlier detection and treatment<sup>21</sup>, has been reported to result in a  
119 shift towards less advanced clinical presentations, in those MEN1 patients undergoing screening  
120 when compared to those not undergoing screening, with lower rates of malignant PNETs (0%  
121 versus 14%), metastases (0% versus 7%) and death (0% versus 7%)<sup>22</sup>. PNETs in MEN1 patients,  
122 which are usually diagnosed between <10 and 50 years of age, are frequently multiple, although  
123 small (i.e. <1cm), and occur on a background of diffuse microadenomatosis<sup>23,24</sup>. In addition, PNETs  
124 in MEN1 patients occur concomitantly with other tumours and the associated comorbidities may  
125 also decrease survival rates. Thus, the mean age of death for MEN1 patients with PNETs is 55  
126 years, which is lower than that expected for the general population, and approximately 40% of these  
127 deaths are due to malignant PNETs, which are usually non-functioning PNETs, and are not  
128 associated with a clinical syndrome<sup>19,20</sup>. These non-functioning PNETs in MEN1 patients are  
129 invariably located within the pancreas and ~33% and 15% will be associated with lymph node and  
130 hepatic metastases, respectively<sup>24,25</sup>, and are the commonest cause of death in MEN1 patients, with  
131 5 and 10 year survival being about 75% and 50%, respectively<sup>26-28</sup>. In contrast, non-MEN1 PNETS  
132 are usually solitary pancreatic lesions that are diagnosed between 50-80 years of age, and ~50-75%  
133 of these will be associated with regional lymph node or distant hepatic metastasis at diagnosis<sup>29,30</sup>.  
134 These differences between MEN1 and non-MEN1 PNETs make it difficult to extrapolate results of  
135 treatment outcomes from studies of non-MEN1 PNETs to MEN1 PNETs<sup>14,21</sup>.

136

137 **CURRENT THERAPIES FOR PNETS IN NON-MEN1 PATIENTS AND THEIR**  
138 **REPURPOSING FOR PNETS IN MEN1 PATIENTS**

139 Current therapies for PNETs in non-MEN1 patients include medical drugs, surgery, and radiology  
140 interventions (Figure 1), which have been repurposed for treatment of PNETs in MEN1 patients,  
141 despite a lack of their formal evaluation in MEN1 patients. Thus, the current treatments for PNETs  
142 in MEN1 are similar to those for PNETs in non-MEN1 patients, and evidence for the effectiveness  
143 of these treatments comprise anecdotal case reports or small case series. These treatments and their  
144 limitations will be briefly reviewed.

145

## 146 **Medical Therapies**

147 Medical therapies for PNETs can be broadly divided into biotherapies, which target tumour-specific  
148 receptors and intracellular pathways, or chemotherapies, which generally target cell division (Table  
149 1 and Figure 1).

150

## 151 ***Biotherapies***

152 Biotherapies for PNETs can be hormonal, which are based on somatostatin (a peptide hormone that  
153 inhibits release of other hormones, cell proliferation and angiogenesis<sup>31</sup>), or targeted to tumour-  
154 specific molecular changes (e.g. in receptors and signalling pathways) that help the tumours to grow  
155 and spread, and these include: mechanistic target of rapamycin (mTOR) signalling inhibitors,  
156 receptor tyrosine kinase (RTK) inhibitors, and antibodies targeting the vascular endothelial growth  
157 factor (VEGF) or its receptor (VEGFR) (Table 1 and Figure 1).

158

159 *Somatostatin analogues* (e.g. octreotide and lanreotide) have been used to control excessive  
160 hormone secretion and for their potential anti-proliferative effects in patients with low-grade  
161 (Ki67<5%) PNETs that express somatostatin receptors (SSTRs), which are G-protein-coupled  
162 receptors (GPCRs)<sup>32-35</sup>. There are 5 SSTRs (SSTR<sub>1-5</sub>) and PNETs may express all 5 subtypes,

163 although ~80% of PNETs will predominantly express SSTR<sub>2</sub>, for which octreotide and lanreotide  
164 have high affinities<sup>32</sup>. Treatment with lanreotide has been reported to result in a ~50% reduction in  
165 the risk of disease progression and a prolonged progression-free survival (PFS) (median in  
166 lanreotide treated group not reached versus median in placebo treated group of 18 months, p<0.001)  
167 in non-MEN1 patients with treatment naive well-differentiated advanced gastroenteropancreatic  
168 NETs (Supplementary Table 1)<sup>34,36,37</sup>. However, this trial, which studied 204 patients did not  
169 contain any MEN1 patients, who were excluded<sup>36</sup>. However, a retrospective evaluation of 40  
170 MEN1 patients with dudeno-pancreatic NETs who were treated with long-acting octreotide,  
171 reported tumour response in 10%, stable disease in 80%, and progression of disease in 10% of  
172 patients over 12-15 months of treatment<sup>38</sup>, thereby suggesting that somatostatin analogue treatment  
173 may have some anti-oncogenic benefits for MEN1 patients.

174

175 Approximately 15% of PNETs have somatic mutations of genes associated with the mTOR  
176 pathway<sup>8</sup>, which regulates cell proliferation and growth (Figures 1 and 3), and the *mTOR inhibitor*  
177 everolimus has been reported to increase PFS from ~6 to 11 months in patients with advanced  
178 NETs, including PNETs<sup>39-42</sup>. Details of the occurrence of MEN1, *MEN1* mutations, or mutations of  
179 the components of the mTOR pathway in the 401 patients in this trial were not provided<sup>39-41</sup>, and  
180 thus it remains to be established whether such mutations may be associated with any differential  
181 responses to mTOR inhibitor therapy. PNETs are highly vascular and frequently express  
182 VEGFRs<sup>43,44</sup>, and *RTK inhibitors*, e.g. sunitinib, which targets VEGFRs, and platelet-derived  
183 growth factor receptors (PDGFRs) have also been reported to increase PFS from ~5.5 to 11.4  
184 months, in patients with PNETs<sup>45</sup>. However, the efficacy of sunitinib in treating PNETs in MEN1  
185 patients remains to be evaluated, because this trial, which comprised a total of 171 patients,  
186 included only 2 MEN1 patients and both of these were not in the treatment arm of the study<sup>45</sup>.



187 Pazopanib, another RTK inhibitor, has also been reported, in a phase 2 trial, to result in response  
188 and disease control rates of ~20% and >75%, respectively of non-MEN1 patients with metastatic  
189 gastroenteropancreatic NETs<sup>46</sup>.

190

191 *Combination therapy using biotherapies* that act on the different receptors and signalling pathways  
192 that regulate PNET proliferation and growth have been reported to result in beneficial effects and  
193 improved outcomes, when compared to monotherapy for gastro-intestinal NETs and PNETs that  
194 occurred in non-MEN1 patients, or in patients whose MEN1 status was unknown (Supplementary  
195 Table 1). For example, combined use of: 1) octreotide with everolimus (in a phase 3 study) or  
196 pazopanib (in a phase 3 study), increased PFS or PNET responses, respectively<sup>47,48</sup>; 2)  
197 temsirolimus, a mTOR inhibitor, with bevacizumab, a monoclonal antibody directed at VEGF, in a  
198 phase 2 study, reduced tumour size and increased PFS in >40% and ~80% of patients, respectively,  
199 with PNETs<sup>49</sup>; 3) bevacizumab, everolimus and octreotide, in a phase 2 study, increased PFS more  
200 than everolimus and octreotide in patients with PNETs<sup>50,51</sup>; and 4) pasireotide, a somatostatin  
201 analogue which targets SSTR<sub>2</sub> and SSTR<sub>5</sub>, which in a phase 2 monotherapy study seemed to inhibit  
202 growth of metastatic NETS, including PNETs<sup>52</sup>, but was associated, in phase 1 and 2 trials, with  
203 hyperglycaemia and bradycardia in ~80% and 30% of patients, respectively<sup>52,53</sup> when used with  
204 everolimus decreased tumour size in >80% of the patients with unresectable or metastatic PNETs,  
205 in a phase 1 trial<sup>54</sup>. However, some targeted therapies and their combinations are not always  
206 successful, and examples include: 1) lanreotide with interferon alpha (IFN $\alpha$ ), which induces cell  
207 cycle arrest and also has anti-angiogenic effects in a randomized trial treating metastatic  
208 gastroentero pancreatic NETs, resulted only in antiproliferative results that were similar to  
209 treatment with lanreotide or IFN $\alpha$ <sup>55</sup>; 2) everolimus and octreotide combined with a monoclonal  
210 antibody to the insulin-like growth factor-1 receptor (IGF1R), which is a RTK that is expressed in

211 PNETs and that promotes cell proliferation by activation of PI3K/AKT signalling and subsequently  
212 mTOR activity and whose blockade impairs NET cell growth, in combined therapy, during phase 1  
213 and 2 trials, were found to be unsafe and to not result in a PNET response<sup>56-60</sup>; 3) octreotide and  
214 bevacizumab, with pertuzumab, a dimerization inhibitor of the epidermal growth factor receptor  
215 (HER1), which is overexpressed in NETs, in a phase 2 study, failed to result in adequate response  
216 rates in patients with advanced PNETs<sup>61</sup>; and 4) dactosilib, an inhibitor of PI3K and the mTOR  
217 complex 2, which is not inhibited by everolimus, in a phase 2 study, failed to improve outcome in  
218 patients with PNETs that were inadequately treated with everolimus<sup>62</sup>.

219

220 The use of these biotherapies varies in different international centres, and generally the somatostatin  
221 analogues (octreotide and lanreotide), the mTOR inhibitor (everolimus), and the RTK inhibitor  
222 (sunitinib) are accepted for treatment of PNET.

223

## 224 ***Chemotherapy***

225 Chemotherapy is reserved to treat patients who have PNETs associated with: metastases; a high  
226 tumour burden; a high proliferative index (i.e. Ki67 >5% or mitosis >5/10 per high powered field);  
227 rapid tumour progression; and/or symptoms not controlled by biotherapy<sup>14,63,64</sup>. Chemotherapy  
228 drugs can be classified in 6 categories (Tables 1 and Supplementary Table 2, and Figure 1), and  
229 drugs from each of these, except the non-classical compounds, have been used to treat  
230 PNETs<sup>14,63,64</sup>. These chemotherapy drugs have actions at different stages of mitosis and include:  
231 *alkylating agents* (e.g. streptozocin, temozolomide and cisplatin), which are cell cycle-independent  
232 drugs that covalently bind to DNA via their alkylating groups, disrupt DNA replication and cause  
233 apoptosis; *anti-microtubule agents* (e.g. etoposide and docetaxel) that disrupt the function of  
234 microtubules, which are required for cell division; *topoisomerase inhibitors* (e.g. doxorubicin and

irinotecan) that prevent the normal unwinding of DNA that is required during replication or transcription, by blocking the activity of topoisomerase enzymes, which produce single or double-strand breaks in DNA, thereby reducing the tension in DNA strands adjacent to the unwound double-stranded DNA helix; *antimetabolites* (e.g. 5fluorouracil (5FU) and its pro-drug capecitabine, and gemcitabine), which are cell cycle dependent, block enzymes required for DNA synthesis or are incorporated into DNA, thereby damaging it and inducing apoptosis; and *cytotoxic antibiotics* (e.g. actinomycin D, mitomycin C, doxorubicin and mixoxantrone), which either alkylate DNA, become intercalated into DNA, or generate highly reactive free radicals that damage intracellular molecules or inhibit topoisomerases.

244

Combination therapy using cytotoxic drugs that act on different cell division components result in better tumour responses, when compared to monotherapy, in patients with PNETs, and streptozocin- and temozolomide-based regimes have been reported to yield substantially higher response rates. For example, studies in the 1990's reported that monotherapy with streptozocin (an alkylating agent) and 5FU (an antimetabolite) resulted in PNET response rates of ~40% and 35%, respectively<sup>65</sup>; and that combined therapies with streptozocin and 5FU or doxorubicin (a topoisomerase inhibitor) resulted in response rates of >60% and ~70%, respectively<sup>66</sup>. However, these early studies used non-standard response rates, and more recent studies using World Health Organisation (WHO) criteria and response evaluation criteria in solid tumours (RECIST) have reported lower PNET response rates of ~35% for streptozocin-based combined regimes<sup>67</sup>. Temozolomide (an alkylating agent) monotherapy resulted in PNET response rates of <10%<sup>68</sup>, whereas temozolomide-based combined therapies with: capecitabine (an antimetabolite) was reported from a retrospective review to result in PNET response rates of ~60%<sup>69</sup>; everolimus, in a phase1/2 study, was reported to result in tumour regression in 40% of patients with metastatic or

259 locally unresectable PNETs<sup>70</sup>; and with bevacizumab in combination with streptozocin or  
260 capecitabine, in phase 2 studies, improved PFS in patients with metastatic NETs, including  
261 PNETs<sup>71-73</sup>. None of these studies reported on the occurrence of MEN1 in the patients  
262 (Supplementary Table 1).

263

## 264 **Surgery**

265 The ideal treatment for a non-metastatic single PNET is surgical excision, as this offers the only  
266 potentially curative treatment. However, surgery is often not successful in MEN1 patients compared  
267 to that in non-MEN1 patients, for the following reasons: first, MEN1-PNETs are usually multiple  
268 (Figure 2C) with sizes varying from micro-adenomas to larger than 4cm<sup>4,24,74,75</sup> thereby making it  
269 difficult to achieve a successful surgical cure; and second, occult metastatic disease may be  
270 present<sup>76</sup>. In addition, the clinical behaviour of these PNETs also varies and it is generally  
271 considered that all macroscopic PNETs are potentially malignant, although the aggressiveness of an  
272 individual PNET cannot be accurately predicted by tumour size, radiological features, or hormone  
273 production<sup>77-79</sup>. However, studies have shown that most microadenomas are stable and infrequently  
274 increase in size; less than 20% of macroadenomas smaller than 2cm will increase in size over 10  
275 years; approximately 50-70% of PNETs between 2-3cm will be associated with lymph node  
276 metastasis; and that 25-40% of PNETs greater than 4cm will be associated with hepatic  
277 metastasis<sup>19,24,29,80,81</sup>. Survival in patients with MEN1 correlates with non-metastatic disease; for  
278 example, survival at 15 years in MEN1 patients with gastrinomas smaller than 2.5cm in size that are  
279 associated with non-metastatic disease or metastatic disease, is reported to be 100% and 50%,  
280 respectively<sup>80-83</sup>. Thus, the occurrence of multiple PNETs and their varied and unpredictable  
281 malignant potential in MEN1 patients pose difficulties for the timing and extent of curative surgery.  
282 As a result of this MEN1 patients with PNETs frequently require additional non-surgical

283 treatments, such as biotherapies, chemotherapy (see above) or radiological-based therapies (see  
284 below).

285

## 286 **Radiological Therapies**

287 Radiological therapies for PNETs include external beam radiotherapy, peptide receptor radionuclide  
288 therapy (PRRT), and interventional radiology (Table 1 and Figure 1). PNETs are not sensitive to  
289 external beam radiotherapy and PRRT is the preferred treatment<sup>14,63,64</sup>.

290

### 291 ***Peptide receptor radionuclide therapy (PRRT)***

292 PRRT is usually based on a somatostatin analogue (e.g. octreotide, octreotate, dototate, and dota-  
293 toc) that has been labelled with a  $\beta$ -emitting nuclide in the form of either <sup>177</sup>Lutetium or <sup>90</sup>Yttrium.  
294 After binding to SSTRs (Figure 1) and internalisation of the receptor complex, the ionising  
295 radiation is released, causing damage to tumour DNA and cell death. To date, the only results  
296 assessing the efficacy of PRRT in PNETs are from cohort studies of patients, which have also  
297 included patients with other gastro-intestinal NETs and metastatic NETS<sup>84-89</sup>. These studies, which  
298 do not comment on the occurrence of MEN1 in the patients, have assessed the effects of  
299 <sup>177</sup>Lutetium- or <sup>90</sup>Yttrium-octreotide, and have reported objective response rates of ~20-60%<sup>84,86-88</sup>,  
300 PFS of 20-34 months<sup>86,88</sup>, and an overall survival of 53 months<sup>86</sup>. Moreover, combining <sup>177</sup>Lutetium  
301 and <sup>90</sup>Yttrium nuclides increased survival in a nonrandomized trial in patients with NETs including  
302 PNETs<sup>89</sup>, and the combination of <sup>177</sup>Lu-octreotate, capecitabine and temozolomide, in a phase1/2  
303 study, resulted in complete or partial response in >50% of the patients with in advanced NETs  
304 (including PNETs)<sup>85</sup>.

305

### 306 ***Interventional Radiology***

Interventional radiology (Table 1 and Figure 1) using radiofrequency ablation (RA), transarterial embolization (TAE), transarterial chemoembolisation (TACE), and selective internal radiation therapy (SIRT) have been shown to be effective treatments in selected cases of PNETs occurring in non-MEN1 patients, or in whom the MEN1 status of the patient was not reported (Supplementary Table 1). RFA has been reported to be effective in treating primary PNETs in patients, who are unable or unwilling to undergo surgical intervention<sup>90</sup>, or who have solitary PNET hepatic metastases<sup>91</sup>. TAE, which involves systemic infusion of microparticles that cause ischemia and tissue necrosis, is reported to be effective for inoperable primary PNETs<sup>92,93</sup>, and hepatic PNET metastases<sup>94</sup>. Combining TAE of the hepatic artery with sunitinib in patients with PNETs, in a phase 2 study, has been reported to result in >65% PFS, and ~60% overall survival rates<sup>95</sup>. TACE combines TAE with locoregional chemotherapy, and TACE, using doxorubicin-eluting beads, of hepatic metastases from gastrointestinal NETs, of which ~40% were from PNETs, has been shown, in a phase 2 study, to be effective<sup>96,97</sup>, although this was associated with severe adverse events<sup>97</sup>. TACE and TAE have similar antitumour effects, but post-embolisation syndrome is commoner with TACE treatment, and the superiority of TACE over TAE in treating NETs remains unproven<sup>98</sup>. SIRT, which is used to deliver <sup>90</sup>Yttrium-labelled spheres in the hepatic artery to provide local radiotherapy for hepatic metastases, was associated with objective tumour response in ~35% and stable disease in ~55% of patients with NETs (10% of whom had PNETs) after 20 months<sup>99</sup>.

325

These medical and radiological based therapies potentially should be effective in MEN1 patients, but they require formal evaluation, as the effects of comorbidities from other endocrine and non-endocrine tumours in MEN1 patients may affect the outcomes. To date, the majority of trials have either excluded MEN1 patients, or only included occasional MEN1 patients in the non-treatment arm (Supplementary Table 1). Thus, to facilitate re-purposing of these therapies it would be

331 important to undertake their evaluations in MEN1 patients. In addition, new therapeutic modalities  
332 based on the function of menin may help in improving the outcome and prognosis for MEN1  
333 patients.

334

## 335 **EMERGING THERAPIES FOR MEN1-ASSOCIATED PNETS FROM PRECLINICAL** 336 **STUDIES**

337

338 Therapies based on increased understanding of menin and of receptors and signalling pathways in  
339 PNETs (Figure 3) are emerging and assessment of their efficacy have been facilitated by cellular  
340 and *in vivo* models which include conventional and conditional *Men1* knockout mouse models that  
341 develop MEN1-associated tumours, including PNETs<sup>100-109</sup>. Menin is a ubiquitously expressed  
342 protein that functions as a nuclear scaffold protein with roles in transcriptional regulation, genome  
343 stability, cell division, cell cycle control and epigenetic regulation<sup>110-112</sup>, that enable it to influence  
344 pathways of cellular proliferation (Figure 3). For example, menin inhibits: Wnt signalling by  
345 transferring  $\beta$ -catenin from the nucleus, which reduces cell proliferation<sup>110,113</sup>; the activity of JunD  
346 by blocking its phosphorylation and therefore potentially subsequent interaction with co-  
347 activators<sup>111,114</sup>, causing JunD to prevent rather than promote cell growth<sup>115</sup>; and Hedgehog pathway  
348 signalling, which influences several functions including tumorigenesis, by recruitment of a protein  
349 arginine methyltransferase (PRMT5), which inactivates the Hedgehog pathway promotor *Gas1*<sup>116</sup>.  
350 Moreover, menin interacts with the mixed lineage leukaemia protein 1 (MLL1) histone  
351 methyltransferase complex to methylate histone H3 (Lys4), causing chromatin modification and  
352 increased transcriptional activity of genes including cyclin dependent kinase inhibitors p27 and p18,  
353 which are involved in cell cycle regulation<sup>117,118</sup>. Menin also promotes the cytostatic effects of  
354 transforming growth factor-beta (TGF- $\beta$ ) by interaction with the Smad pathway<sup>119-121</sup>, as well as

355 interacting with NF- $\kappa$ B proteins to modulate NF- $\kappa$ B transactivation<sup>122</sup>. In addition, menin has roles  
356 that include interaction with the GTPase K-Ras and sons of sevenless (SOS), which prevents K-  
357 Ras-SOS interaction that is essential for K-Ras activation<sup>123</sup>. Moreover, in murine pancreatic  $\beta$ -cells  
358 menin activates opposing K-Ras pathways that comprise a proliferative pathway, likely via  
359 regulation of MAPK and ERK phosphorylation, and an anti-proliferative pathway via RASSF1A<sup>124</sup>.  
360 Thus, menin is considered to interact with K-Ras to block the MAPK/ERK pathway, thereby  
361 inhibiting proliferation, and menin loss removes this inhibition and leads to increased cell  
362 proliferation<sup>124</sup>. In addition, SSTR modulation of proliferation may also occur through K-Ras  
363 signalling, thereby highlighting the importance of K-Ras signalling in PNETs, and indicating that  
364 menin may play a role in SSTR downstream signalling<sup>125</sup>. Furthermore, menin acts as a suppressor  
365 of ERK-dependent phosphorylation of target proteins<sup>126</sup>, and an inhibitor of the mTOR signalling  
366 pathway, by binding to Akt and preventing its translocation to the plasma membrane<sup>127</sup>. Some  
367 recent therapies emerging from pre-clinical studies that target these menin-specific pathways  
368 (Figure 3) will be discussed, and include *MEN1* gene therapy, epigenetic modulators, Wnt  
369 signalling modulators, anti-angiogenic agents, and use of a somatostatin analogue as a  
370 chemopreventative agent.

371

### 372 ***MEN1* gene therapy**

373 The role of menin as a tumour suppressor was supported by *in vitro* studies which demonstrated  
374 that menin overexpression, by use of recombinant plasmid adenoviral or retroviral vectors, in  
375 menin-null mouse embryonic fibroblasts (MEFs), RAS-transformed NIH3T3 MEFs, and rat  
376 insulinoma cell lines resulted in decreased cell proliferation and increased apoptosis<sup>127-131</sup>. In  
377 addition, injection of RAS-transformed NIH3T3 MEFs that over expressed menin, into athymic  
378 nude mice was associated with reduced tumour growth, further supporting a likely tumour



379 suppressor role for menin *in vivo*<sup>128</sup>. These observations, which showed that over-expression of  
380 menin could reduce proliferation, suggested that *MEN1* gene replacement therapy may be  
381 efficacious, and this was evaluated in a mouse model for MEN1, using a recombinant non-  
382 replicating adenoviral serotype 5 vector (rAd5), containing *Men1* cDNA under the control of a  
383 cytomeglavirus promoter (rAd5-MEN1). To establish proof-of-principle for the efficacy of *MEN1*  
384 gene therapy, the rAd5-MEN1 vector was injected into pituitary NETs that developed in  
385 conventional knockout mice lacking one allele of *Men1* (*Men1*<sup>+/-</sup>). This *Men1* gene therapy resulted  
386 in increased menin expression with decreased proliferation of the pituitary NETs, without inducing  
387 an immune response or increased apoptosis<sup>132</sup>. Moreover, the adenoviral gene therapy was not  
388 associated with a higher mortality, and these results indicate the potential utility of *MEN1* gene  
389 replacement therapy for *in vivo* treatment of MEN1-associated NETs<sup>132</sup>. Use of a hybrid adeno-  
390 associated virus and phage (AAVP) vector displaying biologically active octreotide on the viral  
391 surface for ligand-directed delivery of the pro-apoptotic tumour necrosis factor (TNF) transgene to  
392 PNETs developing in a pancreatic specific (*Pdx1-Cre*) *Men1* knockout mouse model, has also been  
393 reported to reduce tumour size, and improve survival of the mutant mice<sup>133</sup>. These results suggest  
394 that systemic, ligand-directed transgene treatment of MEN1-related tumours could evolve as a  
395 novel and effective treatment of MEN1-related tumours.

396

### 397 **Epigenetic modulators**

398 Cancer is associated with alterations in epigenetic mechanisms such as histone modifications and  
399 methylation of DNA, and epigenetic modulators represent a novel class of anti-cancer drugs<sup>134</sup>.  
400 Menin interacts with a number of histone modifying proteins including histone methyltransferase  
401 (MLL1 and PRMT5, Figure 3) and deacetylase complexes (mSin3A-histone deacetylase)<sup>10</sup>, and the  
402 use of epigenetic modulators to treat PNETs was therefore assessed utilising JQ1, an inhibitor of the

403 bromo and extra terminal domain (BET) family of proteins that bind to acetylated histone residues  
404 to promote gene transcription. *In vitro* studies revealed that JQ1 decreased proliferation and  
405 increased apoptosis of PNET and bronchial NET cell lines<sup>135</sup>. These anti-proliferative effects of  
406 JQ1 were associated with: increased numbers of NET cells in G1, and decreased numbers in S and  
407 G2 phases of the cell cycle; and with increased expression of histone 2B, which was likely mediated  
408 through altered activity of BET proteins<sup>135</sup>. Assessment of JQ1 *in vivo*, using a pancreatic  $\beta$ -cell  
409 specific conditional (*RIP2-Cre*) *Men1* knockout mouse model that develops PNETs, revealed that  
410 JQ1 decreased proliferation and increased apoptosis of PNETs. CP103, another BET inhibitor, has  
411 also been reported to reduce PNET proliferation in a human PNET cell line (BON-1) xenograft  
412 mouse model<sup>136</sup>. Thus, epigenetic modulators, e.g. via BET inhibition, may offer potential therapies  
413 for MEN1-associated PNETs.

414

#### 415 **Wnt signalling modulators**

416 Menin inhibits Wnt signalling, as it promotes phosphorylation of  $\beta$ -catenin and its transfer from the  
417 nucleus, which reduces cell proliferation (Figure 3)<sup>110,113</sup>. Moreover, the conditional knockout of  $\beta$ -  
418 catenin in MEN1-deficient PNETs of mice with pancreatic  $\beta$ -cell (*RIP-Cre*) conditional knockout  
419 of menin, decreased the number and size of PNETs, as well as increasing survival<sup>137</sup>. These  
420 findings suggest that modulation of Wnt signalling may represent another approach for treatment of  
421 MEN1 PNETs, and use of a  $\beta$ -catenin antagonist (PKF115-584) decreased PNET cell proliferation  
422 in  $\beta$ -cell menin knockout mice<sup>137</sup>. Thus, Wnt-signalling modulators may provide a novel approach  
423 for treatment of PNETs in MEN1 patients.

424

#### 425 **Anti-angiogenic compounds**

426 The majority of anti-angiogenic compounds block the actions of VEGF, a cytokine that promotes  
427 the growth and survival of blood vessels, and treatment with bevacizumab, an anti-VEGF  
428 monoclonal antibody, in combination with chemotherapy delayed progression of moderately well-  
429 differentiated and advanced gastro-intestinal NETs, which included metastatic well-differentiated,  
430 PNETs<sup>71-73,138</sup>. However, such inhibition of VEGF signalling has been reported to be accompanied  
431 by increased invasiveness and metastasis of cancers and PNETs developing in a transgenic mouse  
432 model that expressed the large T antigen (Tag) encoded by the simian virus 40 (SV40) under the  
433 control of the rat insulin promoter (RIP) and had been rendered null for the recombinase activator  
434 gene Rag1 (RIP-Tag2,Rag1 knockout mice)<sup>139</sup>. This progression of the cancers and PNETs was  
435 reported to be associated with increased tumour hypoxia and expression of pro-angiogenic factors  
436 including VEGFA, members of the fibroblast growth factor (FGF) family, Ephrin A1, and c-Met  
437 activation<sup>139</sup>. In addition, treatment with RTK inhibitors (e.g. sunitinib), which act on VEGF and  
438 PDGF receptors, decrease growth of PNETs in mice, but increase tumour invasiveness and liver  
439 metastases, possibly by upregulation of proangiogenic factors including FGFs<sup>140</sup>, and thus targeting  
440 FGFs in addition to VEGF and PDGF may improve efficacy<sup>141</sup>. Furthermore, combining sunitinib,  
441 or an anti-VEGF antibody, with inhibitors (e.g. PF-04217903 or PF-0241066 (crizotinib)) of c-Met,  
442 which promotes cell proliferation, invasion and metastasis, prevented these increases in tumour  
443 aggressiveness without any impairment to restriction of tumour growth<sup>142</sup>. These findings indicate  
444 that the invasion and metastasis that are promoted by selective inhibition of VEGF signalling, may  
445 be reduced by combined treatment with a c-met inhibitor. In addition, inhibition of nitric oxide  
446 (NO) synthase may have a role, as demonstrated by use of L-arginine methyl ester (L-NAME), a  
447 NO synthase inhibitor, which in *ex vivo* treatment of PNETs from conventional *Men1*<sup>+/-</sup> knockout  
448 mice, caused impaired blood perfusion and increased constriction of the tumour supplying  
449 arterioles<sup>143</sup>. Trombospondin-1 (Figure 3) analogues may also have a role, as administration of

450 thrombospondin-1 suppressed angiogenesis and tumour growth of PNETs in a transgenic mouse  
451 model (RIP-Tag2)<sup>144</sup>, and it is of interest to note that menin interacts with Smad3 which is  
452 downstream of the thrombospondin-1-transforming growth factor beta (TGFβ) signalling  
453 pathway<sup>145</sup>.

454

#### 455 **Use of somatostatin analogue for tumor chemoprevention**

456 Cancer chemoprevention involves the chronic administration of a synthetic, natural, or biological  
457 agent to reduce or delay the occurrence of tumours, and its value has been demonstrated in breast,  
458 prostate, and colon cancer trials<sup>146-149</sup>. Currently, individuals with a *MEN1* mutation are entered into  
459 a screening programme for MEN1-associated tumours, including PNETs, commencing from the  
460 first or second decade of life<sup>21</sup>. This approach will detect tumours early, thereby facilitating earlier  
461 treatment (e.g. surgery), but will not prevent or delay the occurrence of tumours, i.e.  
462 chemoprevention. Somatostatin analogues may have a potential role in chemoprevention of MEN1-  
463 associated NETs as they have been shown to have antiproliferative, and antiseecretory effects on  
464 NETs<sup>36,37,150,151</sup>. Thus, treatment with pasireotide, which is a multiple-receptor-targeted  
465 somatostatin analogue that acts via SSTR<sub>1,2,3</sub> and SSTR<sub>5</sub><sup>32</sup>, decreased proliferation and increased  
466 apoptosis of PNETs in *Men1*<sup>+/-</sup> and *Pdx-Cre Men1* knockout mutant mice<sup>152,153</sup>. Pasireotide also  
467 decreased proliferation and increased apoptosis of pituitary NETs, in *Men1*<sup>+/-</sup> mice, as well as  
468 increasing survival of the *Men1*<sup>+/-</sup> mutant mice<sup>152,153</sup>. In addition to these anti-proliferative and pro-  
469 apoptotic effects, pasireotide was also found to inhibit the development of PNETs in the *Men1*<sup>+/-</sup>  
470 mutant mice<sup>152</sup>. Thus, PNET occurrence was significantly lower in *Men1*<sup>+/-</sup> mice treated with  
471 pasireotide when compared to *Men1*<sup>+/-</sup> mice treated with control phosphate buffered saline (PBS)  
472 (87% of pasireotide-treated versus ~97% of PBS-treated mice, p<0.05)<sup>152</sup>. Moreover, the mean  
473 number of PNETs per pancreas was also significantly lower in the pasireotide-treated *Men1*<sup>+/-</sup> mice

474 when compared to PBS-treated *Men1*<sup>+/-</sup> mice (2.36± 0.25 in pasireotide-treated versus 3.72± 0.32,  
475 p<0.001). These findings, which indicate that pasireotide-treatment resulted in fewer *Men1*<sup>+/-</sup> mice  
476 with PNETs who also had fewer PNETs per pancreas, when compared to PBS-treated *Men1*<sup>+/-</sup> mice,  
477 are consistent with a lower development of new NETs, in pasireotide treated *Men1*<sup>+/-</sup> mice<sup>152</sup>.  
478 Moreover, the pasireotide treated *Men1*<sup>+/-</sup> mice appeared healthier and had increased survival than  
479 the placebo treated *Men1*<sup>+/-</sup> mice, and adverse effects from the pasireotide treatment were not  
480 detected<sup>152</sup>. These findings, suggest that somatostatin analogues may have a chemopreventative role  
481 in the treatment of MEN1-associated PNETs in humans, and two studies have reported that  
482 somatostatin analogues have anti-proliferative actions in PNETs of MEN1 patients<sup>38,154</sup>. In one  
483 study octreotide was given to MEN1 patients to treat duodeno-pancreatic NETs, and retrospective  
484 analysis revealed that 10% of PNETs had tumour response, and that 80% had stable disease<sup>38</sup>; and  
485 in another study, octreotide was given prospectively to 8 MEN1 patients with GEP-NETs, and  
486 found to be safe and decrease gastro-intestinal hormone secretion, and to be associated with stable  
487 PNET disease<sup>154</sup>.

488

## 489 CONCLUSIONS

490

491 MEN1 is an autosomal dominant disorder characterised by the combined occurrence of tumours in  
492 different endocrine glands. The associated hypersecretion of hormones and malignant potential of  
493 these tumours severely reduces life expectancy for MEN1 patients. Current medical, surgical and  
494 radiological treatments for MEN1 patients, which are based on their effectiveness in treating  
495 endocrine tumours in non-MEN1 patients, are associated with inferior outcomes. MEN1 is caused  
496 by mutations of the *MEN1* gene, encoding the tumour suppressor protein, menin, and increased  
497 understanding of the role of menin in tumourigenesis and cell proliferation, has enabled targeted

therapies to be identified. These new treatments, which have been evaluated in pre-clinical studies include: adenoviral MEN1 gene replacement therapy; epigenetic modulators; Wnt pathway-targeting  $\beta$ -catenin antagonists; thrombospondin-1 analogues; and a multiple-receptor-targeted somatostatin analogue. Clinical evaluation of such emerging treatments targeting menin-associated pathways may provide new therapies for improving outcomes, and life expectancy in MEN1 patients.

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958 **Key points**

- 959 • Patients with MEN1 may develop hormone secreting and non-secreting tumours in
- 960 endocrine organs including the pancreas, which decreases their life expectancy substantially.
- 961 • MEN1-related tumours are difficult to treat due to differences in growth potential,
- 962 concomitant development of other tumours, and relative insensitivity to treatment.
- 963 • Current medical, surgical and radiological treatments have not been formally assessed in
- 964 MEN1 patients, but instead been used on the basis of their effects on endocrine tumours in
- 965 non-MEN1 patients.
- 966 • Therapies targeting MEN1 tumours are required, and preclinical studies indicate that gene
- 967 therapy, epigenetic modifiers such as bromo- and extra terminal domain (BET) inhibitors,
- 968 which are acetyl code-reader inhibitors, and Wnt pathway and VEGF-signalling antagonists
- 969 may be promising treatments.
- 970 • Chemoprevention aimed at reducing or delaying the occurrence of MEN1-pancreatic
- 971 neuroendocrine tumours may be possible by chronic administration of somatostatin
- 972 analogues, which have anti-proliferative and anti-secretary actions.

973

974 **Boxes**

975 **Box 1. Multiple Endocrine Neoplasia type 1 (MEN1) clinical features and emerging therapies.**

976 *Clinical features and genetics*

977 Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominant disorder characterised by

978 the combined occurrence of tumours of the parathyroids or neuroendocrine tumours (NETs) of the

979 pancreas, pituitary and adrenal. Patients may also develop other endocrine or non-endocrine

980 tumours. The 20-year survival of MEN1 patients is lower than the general population, and >65% of

981 MEN1 patients die from MEN1-related disease. MEN1 is caused by mutations of the *MEN1* gene,

982 encoding the tumour suppressor protein menin, a nuclear scaffold protein with roles in gene  
983 transcription, genome stabilisation, cell cycle and epigenetic regulation.

984

#### 985 ***Current treatments***

986 Current medical, surgical and radiological treatments, have not been evaluated in MEN1 patients,  
987 but instead successful treatments for endocrine tumours in non-MEN1 patients have been  
988 extrapolated as treatments for MEN1 patients. However, the outcome of such treatments in MEN1  
989 patients are greatly inferior to those in non-MEN1 patients. This may be due to the: concomitant  
990 occurrence of multiple tumours in different glands; presence of occult metastatic disease; associated  
991 co-morbidities that may decrease survival rates; and the younger onset that may result in larger and  
992 more aggressive tumours that are resistant to treatment. Improved treatments for MEN1 tumours are  
993 required.

994

#### 995 ***Emerging therapies***

996 Increased understanding of the role of menin in cell proliferation has enabled targeted therapy of  
997 endocrine tumours in mouse models for NETs with *MEN1* mutations. These include: adenoviral  
998 *MEN1* gene replacement therapy; a hybrid adeno-associated virus and phage vector containing a  
999 ligand motif and tumour necrosis factor transgene for tumour directed therapy, and inducing  
1000 apoptosis; epigenetic modulators; Wnt pathway targeting  $\beta$ -catenin antagonists; Thrombospondin-1  
1001 analogues; receptor tyrosine kinase inhibitors; mammalian target of rapamycin (mTOR) inhibitors;  
1002 and somatostatin analogues, which target a broader spectrum of receptors and may have a potential  
1003 role in tumour chemoprevention.

1004

1005

1006 *Author Biographies*

1007 **Morten Frost** has been a clinician at the Endocrine Research Unit, University of Southern  
1008 Denmark, since 2003, and received his Ph.D. from the University of Southern Denmark in 2011. In  
1009 2016, he visited Professor Rajesh Thakker's laboratory at the University of Oxford to research the  
1010 genetics of neuroendocrine tumour patients, as well as calcium sensing receptor signalling. His  
1011 research currently involves investigating monogenic bone diseases and calcium metabolic disorders.

1012

1013

1014 **Kate Lines** received her PhD in Molecular Oncology from the Barts Cancer Institute, Queen Mary  
1015 University of London, UK in 2011, and is currently a postdoctoral research assistant in the  
1016 laboratory of Professor Raj Thakker at the University of Oxford. Her research currently focuses on  
1017 elucidating the epigenetic mechanisms occurring in neuroendocrine tumours, and targeting these  
1018 mechanisms to develop novel therapeutic approaches.

1019

1020 **Rajesh Thakker** is the May Professor of Medicine at the University of Oxford, and a Fellow of the  
1021 Royal Society. He has pursued molecular, cellular, and physiological analyses of >15 disorders,  
1022 with identification of their defective genes and functional studies that explain the disease  
1023 phenotypes. This has resulted in the elucidation of: molecular mechanisms of endocrine tumour  
1024 formation and potential new therapeutic targets; signalling and regulatory pathways downstream of  
1025 the calcium-sensing receptor, and molecular aspects of renal tubular physiology. He was the lead  
1026 author for the recently published clinical guidelines for multiple endocrine neoplasia type 1  
1027 (MEN1).

1028

1029 **Table 1.** Current treatment options for pancreatic NETs (PNETs)  
 1030

Medical
<b>Biotherapy</b> <ul style="list-style-type: none"> <li>Somatostatin analogues e.g. octreotide, lanreotide, pasireotide</li> <li><math>\alpha</math>-interferon</li> <li>Mechanistic target of rapamycin (mTOR) inhibitors e.g. everolimus</li> <li>Receptor tyrosine kinase (RTK) inhibitors, including PDGFR<sup>a</sup> and VEGFR<sup>a</sup> inhibitors e.g. sunitinib and sorafenib</li> <li>VEGFA<sup>b</sup> antibodies e.g. bevacizumab</li> </ul>
<b>Chemotherapy</b> <ul style="list-style-type: none"> <li>Alkylating agents<sup>c</sup> e.g. streptozocin, temozolomide, cisplatin</li> <li>Anti-microtubule agents<sup>c</sup> e.g. etoposide, docetaxel</li> <li>Topoisomerase inhibitors<sup>c</sup> e.g. doxorubicin, irinotecan</li> <li>Antimetabolites<sup>d</sup> e.g. 5'fluorouracil (capecitabine<sup>e</sup>), gemcitabine</li> <li>Cytotoxic antibodies<sup>d</sup> e.g. actinomycin D, mitomycin C, doxorubicin, mitoxantrone</li> <li>Non-classical compounds</li> </ul>
Surgery
<b>Curative Cytoreduction</b>
Radiological
<b>Radiotherapy</b> <ul style="list-style-type: none"> <li>External beam</li> <li>Tumour targeted (e.g. Peptide Receptor Radionuclide Therapy (PRRT) using <sup>90</sup>Y-DOTATOC, or <sup>177</sup>Lu-DOTATE)</li> </ul>
<b>Interventional Radiology</b> <ul style="list-style-type: none"> <li>Radiofrequency ablation (RFA)</li> <li>Transarterial embolisation (TAE)</li> <li>Transarterial chemoembolisation (TACE)</li> <li>Selective internal radiation therapy (SIRT)</li> </ul>

1031  
 1032 <sup>a</sup>PDGFR – platelet-derived growth factor receptor; and VEGFR – vascular endothelial growth  
 1033 factor receptor are both TKI inhibitors. Inhibitors may have multiple targets, for example sunitinib  
 1034 and sorafenib inhibit PDGFR and VEGFRs; imatinib inhibits PDGFRs, Abelson murine leukemia  
 1035 viral oncogene homolog 1 (vAbI) and proto-oncogene c-Kit (c-kit); and vandetanib inhibits  
 1036 VEGFRs and epidermal growth factor receptors (EGFRs); <sup>b</sup>VEGFA – vascular endothelial growth  
 1037 factor A; <sup>c</sup>Nuclear targets; <sup>d</sup>cytoplasmic targets; capecitabine is the orally administered pro-drug of  
 1038 5'fluorouracil (5FU).  
 1039

## Figure Legends

**Figure 1.** Current treatments for pancreatic neuroendocrine tumours (PNETs). Treatments are: medical, which includes drugs and antibodies that target different pathways of cancer cells; surgical, i.e. removal or resection of the NET; and radiological, in which particles or high frequency waves are delivered externally or internally (e.g. intra-arterially) to the tumour. SSTR – somatostatin receptor; IFNAR – interferon alpha/beta reception; VEGFR – vascular endothelial growth factor receptor; VEGFA – vascular endothelial growth factor A; RTK – receptor tyrosine kinase; mTOR – mechanistic target of rapamycin.

**Figure 2.** Distribution of endocrine and non-endocrine tumours in MEN1 patients. **(A)** MEN1 patients may develop: endocrine tumours involving the parathyroids (labelled number 1), pancreas (2), pituitary (3), adrenal cortex (4) and medulla (5), gastro-intestinal tract (6), thymus (7) and bronchial tree (8); and non-endocrine tumours such as facial angiofibromas (9), collagenomas (10), lipomas (11) and meningiomas (12). **(B)** Frequencies of MEN1-associated tumours. The most frequently occurring endocrine tumours in MEN1 patients are: parathyroid adenomas, which occur in >95% of patients; pancreatic neuroendocrine tumours (PNETs), which occur in 50-70% of patients, with ~40% of patients having gastrinomas, ~10% having insulinomas, <1% having glucagonomas, <1% having VIPomas, and ~20-50% having PPomas or non-functioning tumours; anterior pituitary tumours, which occur in 20-40% of patients, with ~20% having prolactinomas, ~10% having somatotrophinomas, <5% having corticotrophinomas, and ~5% having non-functioning tumours; and adrenal tumours, which occur in 20-40% of patients, with ~40% having cortical adenomas that are usually non-secreting, but may occasionally secrete glucocorticoids, or aldosterone causing Cushing's or Conn's syndrome, respectively, and <1% having pheochromocytoma tumours arising from the medulla. The most frequently occurring non-

1065 endocrine tumours in MEN1 patients are angiofibromas, collagenomas, and lipomas, which are  
1066 reported to occur in 0-85%, 0-70%, and ~30% of patients, respectively. (C) Magnetic Resonance  
1067 Imaging (MRI) sagittal section of multiple PNETs (indicated by white arrows) in an MEN1 patient.  
1068 (D) MRI sagittal section of a non-MEN1 PNET (the tumour is indicated by a white arrow).

1069

1070

1071

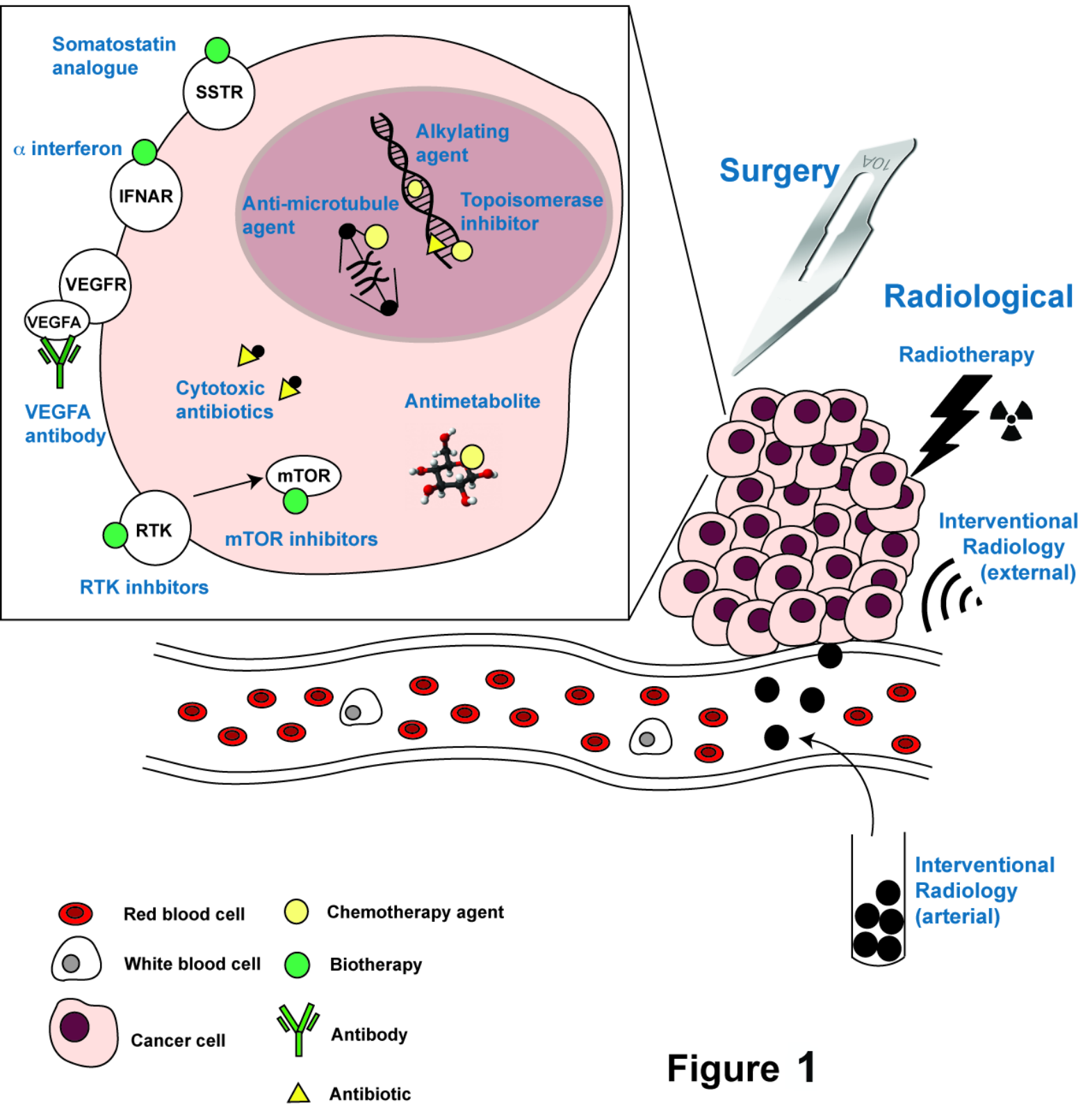
1072 **Figure 3.** Menin-associated pathways with potential targeted therapies. Menin, which is encoded by  
1073 the *MEN1* gene, has roles in the nucleus and cytoplasm, and loss of menin expression (represented  
1074 by grey boxes in the diagram) results in increased cell proliferation by multiple different pathways.  
1075 Thus, in the nucleus, menin: interacts with the transcription factor JunD and the protein arginine  
1076 methyltransferase (PRMT) 5 to repress transcription of target genes, for example *Gastrin* and *Gas1*,  
1077 respectively<sup>115,116</sup>; binds to mixed lineage leukaemia proteins MLL1 and/or MLL2, and Smad3,  
1078 which is a TGF- $\beta$  signalling component, to promote transcription of target genes<sup>117-121</sup>; and  
1079 regulates the Wnt pathway by preventing  $\beta$ -catenin from entering the nucleus and therefore  
1080 preventing transcription of Wnt pathway target genes<sup>110,113</sup>. In the cytoplasm, menin inhibits: the  
1081 mTOR pathway, by binding to Akt, which is downstream of PI3K that is part of the RTK signalling  
1082 pathway, and preventing its translocation to the plasma membrane<sup>127</sup>; and K-Ras induced  
1083 proliferation, by possible inhibition of ERK dependent phosphorylation and prevention of the  
1084 interaction between SOS and K-Ras<sup>123,124</sup>. These advances in understanding the function of menin,  
1085 have helped to identify potential new and targeted therapies (indicated in blue), which include:  
1086 mTOR inhibitors; Wnt pathway inhibitors e.g.  $\beta$ -catenin antagonists; epigenetic modulators; MEN1  
1087 gene replacement; analogues of thrombospondin-1 (TSP1), which interact and alter TGF- $\beta$   
1088 signalling, that includes the menin-interacting protein Smad3; RTK inhibitors; and somatostatin

1089 analogues which act on a broader spectrum of SSTRs. All pathways affect proliferation (shown in  
1090 the cytoplasm only), which involves both nuclear and cytoplasmic process and mechanisms; RTK –  
1091 receptor tyrosine kinase; GF – growth factor; P13K – phosphoinositide 3-kinase; Akt – protein  
1092 kinase B; mTOR – mechanistic target of rapamycin; FRZ – fizzled; MLL – mixed lineage  
1093 leukemia; CDKN – cyclin dependent kinase inhibitor; PRMT5 – protein arginine N-  
1094 methytransferase 5; GAS1 – growth arrest specific 1; SMAD3 – mothers against decapentaplegic  
1095 hormone 3; TGFβ(R) – transforming growth factor beta (receptor); TSP1 – thrombospondin 1;  
1096 SST(R) – somatostatin (receptor); SOS1 – sons of sevenless 1; RASSF1A – ras associated domain  
1097 family member 1 isoform A; MEK – mitogen activated protein kinase kinase; ERK – extra signal-  
1098 related kinase .

1099

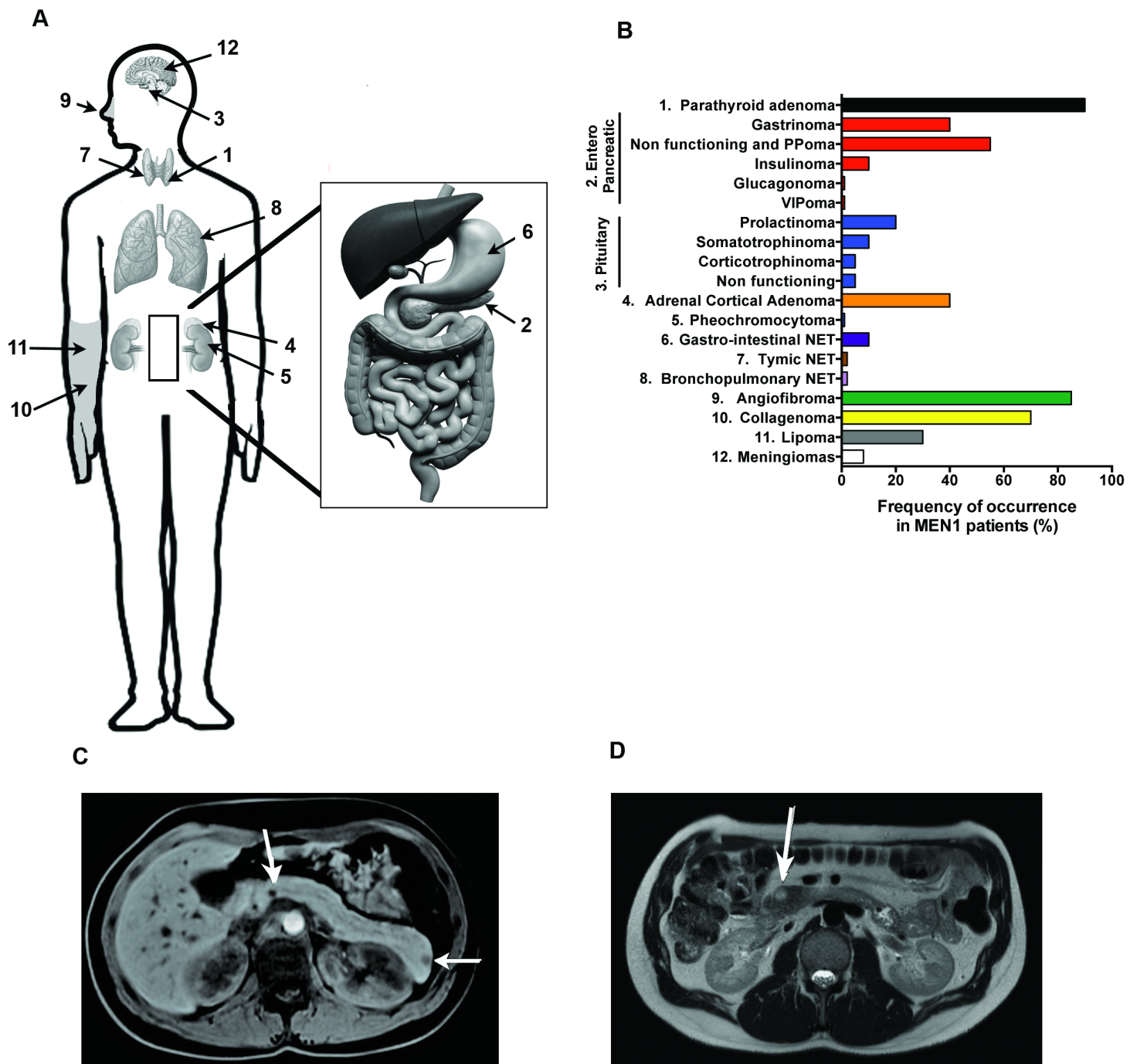
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## Medical Therapy

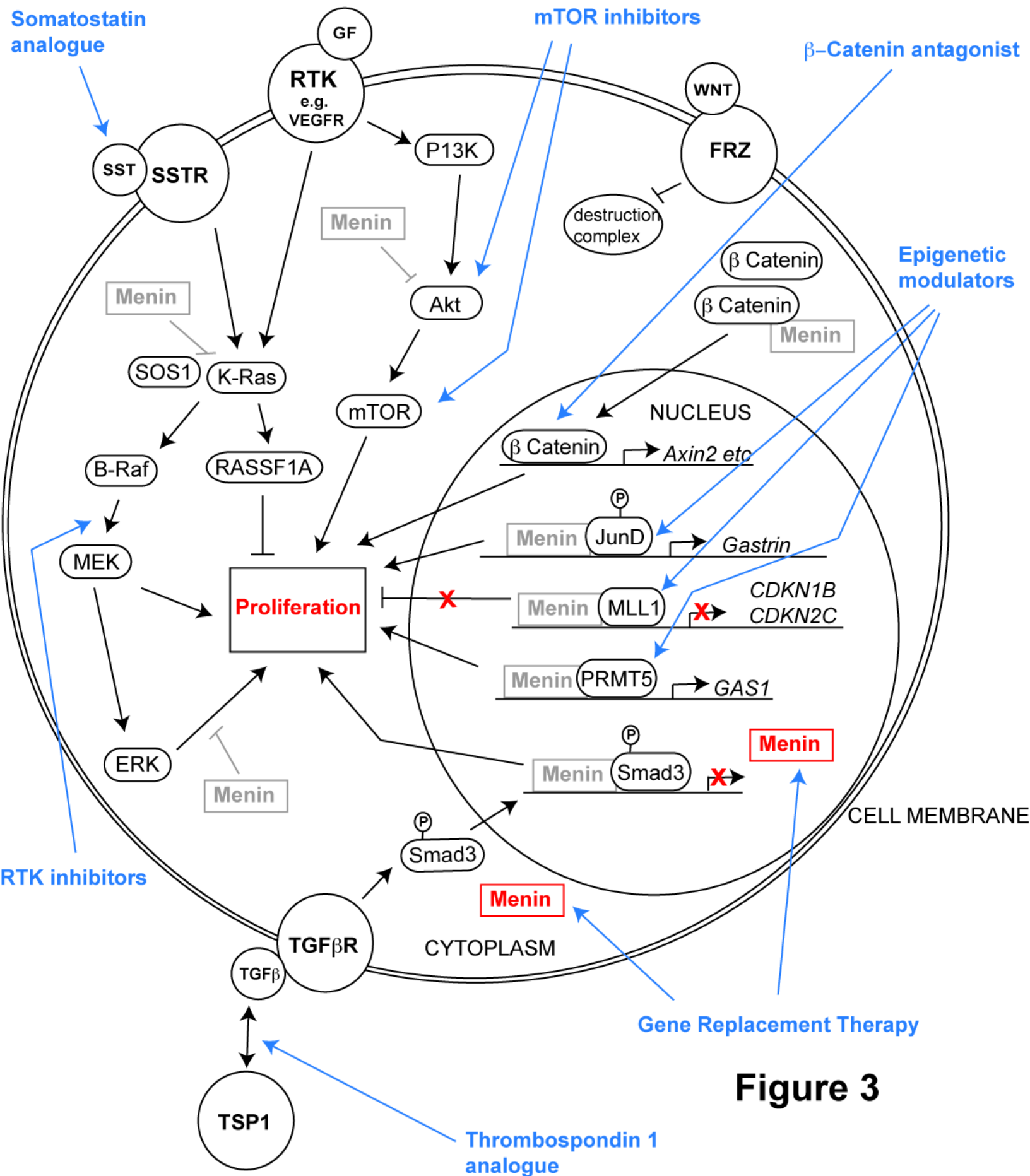


**Figure 1**





**Figure 2**



**Figure 3**

**Supplementary Table 1.** Therapies for non-MEN1 pancreatic neuroendocrine tumours (NETs)  
(published after 2011)

	Tumour type <sup>a</sup>	Intervention	Number of participants/Information available on MEN1 patients/Number of MEN1 patients
<b>Somatostatin analogues</b>			
Caplin <i>et al.</i> <sup>1</sup>	GEPNET	Lanreotide LAR 120 mg or placebo	204/Yes/Excluded from participation
Caplin <i>et al.</i> <sup>2</sup>	GEPNET	Lanreotide LAR 120 mg (continuing or switched from placebo).	88/Yes/Excluded from participation
Martin-Richard <i>et al.</i> <sup>3</sup>	GEP-NET, bronchopulmonary NET and neuroendocrine carcinoma	Lanreotide LAR 120 mg	30/No/Na
Yao <i>et al.</i> <sup>4</sup>	NETs including PNETs	Pasireotide LAR 80 or 120 mg	29/No/NA
Cives <i>et al.</i> <sup>5</sup>	NETs including PNETs	Pasireotide LAR 60 mg	29/No/NA
Wolin <i>et al.</i> <sup>6</sup>	NETs of digestive system including PNETs (two cases only) and carcinoid symptoms	Pasireotide LAR 60 mg or octreotide LAR 40 mg	110/No/NA
Ramundo <i>et al.</i> <sup>7</sup>	Duodeno-PNETs	Octreotide LAR 30mg	20/Yes/20
Cioppi <i>et al.</i> <sup>8</sup>	GEP-NETs	Octreotide LAR 30mg	8/Yes/8
<b>Tyrosine kinase inhibitors</b>			
Raymond <i>et al.</i> <sup>9</sup>	PNET	Sunitinib 37.5 mg or placebo	171/Yes/2
Ahn <i>et al.</i> <sup>10</sup>	GEPNET	Pazopanib 800 mg	37/No/NA
Phan <i>et al.</i> <sup>11</sup>	PNET or carcinoid	Pazopanib 800 mg + octreotide	52/No/NA
Strosberg <i>et al.</i> <sup>12</sup>	PNET or carcinoid	Sunitinib 37.5 mg, following hepatic transarterial embolization	39/No/NA
<b>mTOR inhibitors</b>			
Pavel <i>et al.</i> <sup>13</sup>	NETs including PNETs with carcinoid syndrome	Everolimus 10 mg + octreotide 30 mg or Placebo + octreotide 30 mg	429/No/NA
Anthony <i>et al.</i> <sup>14</sup>	NETs including PNETs with carcinoid syndrome	Assessment of effect of previous treatment with SSAs on outcome of everolimus	Previous SSAs 339/No/NA No previous SSAs 90/No/NA
Yao <i>et al.</i> <sup>15</sup>	PNET	Everolimus 10 mg or placebo	410/No/NA
Lombard-Bohas <i>et al.</i> <sup>16</sup>	PNET (Subgroup analysis of Yao <i>et al.</i> <sup>15</sup> )	Everolimus 10 mg or placebo	410/No/NA
Yao <i>et al.</i> <sup>17</sup>	PNET (Overall survival data from Yao <i>et al.</i> <sup>15</sup> )	Everolimus 10 mg	410/No/NA
Oh <i>et al.</i> <sup>18</sup>	NET, including PNTS, pheochromocytoma or extraadrenal paragangliomas	Everolimus 10 mg	34/No/NA
Chan <i>et al.</i> <sup>19</sup>	PNET	Temozolomide + everolimus 5 mg or temozolomide + everolimus 10 mg	43/No/NA
Chan <i>et al.</i> <sup>20</sup>	PNET or carcinoid	Everolimus + pasireotide	22/No/NA
Chan <i>et al.</i> <sup>21</sup>	PNET or carcinoid	Everolimus 10 mg + sorafenib 400 mg or	21/No/NA

Everolimus 10 mg + sorafenib 600 mg			
<b>Anti-IGF-1 receptor</b>			
Dasari <i>et al.</i> <sup>22</sup>	PNET or carcinoid	Cixutumumab + everolimus 10 mg + octreotide LAR 20 mg	19/Yes/0
Reidy-Lagunes <i>et al.</i> <sup>23</sup>	PNET or carcinoid	Dalotuzumab	25/No/NA
Strosberg <i>et al.</i> <sup>24</sup>	PNET or carcinoid	Ganitumab	60/No/NA
<b>EGFR antibodies and PI3K (mTOR)</b>			
Bendell <i>et al.</i> <sup>25</sup>	PNET or carcinoid	Bevacizumab + pertuzumab + octreotide LAR 30 mg	43/No/NA
Fazio <i>et al.</i> <sup>26</sup>	PNET	Dactosilib	31/No/NA
<b>Peptide receptor radionuclide therapy</b>			
Claringbold <i>et al.</i> <sup>27</sup>	NET including PNET	<sup>77</sup> Lu-octreotate + capecitabine + temozolamide (first escalating doses, then stabile dosis)	35/No/NA
<b>VEGF and VEGFR antibodies</b>			
Chan <i>et al.</i> <sup>28</sup>	PNET or carcinoid	Cabozantinib	61/No/NA (In abstract form)
Ducreux <i>et al.</i> <sup>29</sup>	PNET	Bevacizumab + capecitabine	34/No/NA
Berruti <i>et al.</i> <sup>30</sup>	NET including PNET	Bevacizumab + capecitabine + octreotide	45/No/NA
Chan <i>et al.</i> <sup>31</sup>	PNET or carcinoid	Bevacizumab + temozolamide	34/No/NA
Hobday <i>et al.</i> <sup>32</sup>	PNET	Temsirolimus + bevacizumab	58/No/NA
Kulke <i>et al.</i> <sup>33</sup>	PNET	Everolimus 10 mg + octreotide or everolimus 10 mg + bevacizumab + octreotide	150/No/NA (In abstract form)

GEPNET – Gastroenteropancreatic neuroendocrine tumour; PNET – pancreatic neuroendocrine tumour; NET – neuroendocrine tumour  
 LAR – long acting release formulation  
 NA – not available

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**Supplementary Table 2.** Classification of chemotherapy drugs.

<b>Alkylating agents<sup>a</sup></b>	
	<ul style="list-style-type: none"> <li>• Nitrogen mustards, e.g. <i>cyclophosphamide</i><sup>c</sup>, chlorambucil</li> <li>• Nitrosoureas, e.g. <i>streptozocin</i></li> <li>• Tetrazines, e.g. <i>dacarbazine</i>, <i>temozolomide</i></li> <li>• Aziridines, e.g. thiotepa, mytomycin</li> <li>• Cisplatins, e.g. <i>cisplatin</i>, <i>oxabiplatin</i></li> <li>• Non-classical, e.g. <i>procarbazine</i></li> </ul>
<b>Anti-microtubule agents<sup>a</sup></b>	
	<ul style="list-style-type: none"> <li>• Vincalkaloids, e.g. vinaristine, vinblastine</li> <li>• Taxanes, e.g. paclitaxel, <i>docetaxel</i></li> <li>• Podophyllotoxins, e.g. <i>etoposide</i></li> <li>•</li> </ul>
<b>Topoisomerase inhibitors<sup>a</sup></b>	
	<ul style="list-style-type: none"> <li>• <i>Doxorubicin</i></li> <li>• <i>Etoposide</i></li> <li>• <i>Irinotecan</i></li> </ul>
<b>Cytotoxic antibodies<sup>b</sup></b>	
	<ul style="list-style-type: none"> <li>• Anthracyclines, e.g. <i>doxorubicin</i></li> <li>• Bleomycins</li> <li>• <i>Mitomycin C</i></li> <li>• <i>Acinomycin D</i></li> <li>• <i>Mitoxantrone</i></li> </ul>
<b>Antimetabolites<sup>b</sup></b>	
	<ul style="list-style-type: none"> <li>• Antifolates, e.g. methotrexate</li> <li>• Fluoropyrimidines, e.g. <i>fluorouracil</i>, <i>capecitabine</i></li> <li>• Deoxynucleoside analogues, e.g. <i>gemcitabine</i></li> <li>• Thiopurines, e.g. mercaptopurine</li> </ul>
<b>Non classical compounds</b>	

<sup>a</sup>Nuclear targets; <sup>b</sup>cytoplasmic targets; <sup>c</sup>drugs shown in italics have been used to treat gastroenteropancreatic neuroendocrine tumours (GEPNETs).