Molecular targeted therapies in adrenal, pituitary and parathyroid malignancies

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Abstract

Tumourigenesis is a relatively common event in endocrine tissues. Currently, specific guidelines have been developed for common malignant endocrine tumours, which also incorporate advances in molecular targeted therapies (MTT), as in thyroid cancer and in gastrointestinal neuroendocrine malignancies. However, there is little information regarding the role and efficacy of MTT in the relatively rare malignant endocrine tumours mainly involving the adrenal medulla, adrenal cortex, pituitary, and parathyroid glands. Due to the rarity of these tumours and the lack of prospective studies, current guidelines are mostly based on retrospective data derived from surgical, locoregional and ablative therapies, and studies with systemic chemotherapy. In addition, in many of these malignancies the prognosis remains poor with individual patients responding differently to currently available treatments, necessitating the development of new personalised therapeutic strategies. Recently, major advances in the molecular understanding of endocrine tumours based on genomic, epigenomic, and transcriptome analysis have emerged, resulting in new insights into their pathogenesis and molecular pathology. This in turn has led to the use of novel MTTs in increasing numbers of patients. In this review, we aim to present currently existing and evolving data using MTT in the treatment of adrenal, pituitary and malignant parathyroid tumours, and explore the current utility and effectiveness of such therapies and their future evolution.

Introduction

There are numerous specific guidelines developed for the relatively common malignant endocrine tumours such as thyroid cancer and gastrointestinal neuroendocrine tumours (NETs) (Haugen et al. 2016, Pavel et al. 2016), but there is a paucity for other less common endocrine carcinomas involving the parathyroids, adrenals, and the pituitary gland. Parathyroid tumours have an incidence of 80 and 36 per 100,000 population in women and
men aged 50–59 years, respectively (Yeh et al. 2013), and occasionally can be multiple. Similarly, pituitary and adrenocortical tumours have a prevalence of 16.7% (range 14.4–22.5) and 4% (range 1.0–8.7), respectively, the latter increasing with age (Ezzat et al. 2004, Zeiger et al. 2009, Fassnacht et al. 2016). Tumours derived from the adrenal medulla occur less commonly with a prevalence of 1/2500–6500, (Kantorovich et al. 2015), although autopsy series have revealed a prevalence of 0.05–0.1% (McNeil et al. 2000, Lenders et al. 2014). Adrenocortical carcinomas (ACCs) account for up to 11% (range 1.2–12%) of adrenocortical tumours, whereas up to 25% of phaeochromocytomas (PCs) may become malignant (Ayala-Ramirez et al. 2011, Fassnacht et al. 2016). Approximately, 0.2% of pituitary tumours can develop cranial and extra-cranial metastases (Kaltsas et al. 2005a, Heaney et al. 2011), whereas the incidence of parathyroid carcinomas is estimated at less than 1% in patients with primary hyperparathyroidism, although studies from Japan have reported a higher incidence of 5% (Hamill et al. 2002, Givi & Shah 2010, Mohebati et al. 2012).

Currently, the optimal management of malignant tumours originating from the pituitary, parathyroid, and adrenal glands is not well defined. The standard treatments for these tumours include surgery, locoregional and ablative therapies, administration of somatostatin analogues (SSAs), radionuclides and systemic chemotherapy, albeit with limited efficacy, necessitating the development of more effective treatments.

Recent advances in our understanding of the genetic pathways implicated in the pathogenesis of these tumours are expected to lead to a more personalised approach directed at the specific molecular aberrations. The most common pathways involved in endocrine tumour pathogenesis are the phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and the RAS-mitogen-activated protein kinase (MAPK) cascades, both downstream of growth factor receptors such as the insulin-like growth factor-1 receptor (IGF1-R) (Nölting & Grossman 2012, Ribeiro et al. 2012, Favier et al. 2015) (Fig. 1A and B). Angiogenesis stimulation also seems to play a major role in tumourigenesis, with vascular epithelial growth factor receptors (VEGF-Rs), platelet-derived growth factor receptor (PDGF-Rs), endothelial growth factor receptors (EGF-Rs), and tyrosine kinase inhibitors (TKIs) being the most studied targeted pathways in preclinical and clinical studies (Hay & Sonenberg 2004, O’Reilly et al. 2006, De Martino et al. 2014).

In this review, we aim to present available novel and evolving in vitro and in vivo data on molecular pathways involved in the malignant transformation of adrenal malignancies, pituitary and parathyroid carcinomas, as well as evidence from current clinical applications of molecular targeted treatment (MTT).

### Phaeochromocytomas and paragangliomas

**Introduction**

Phaeochromocytomas (PCs) arise from the adrenal chromaffin cells, whereas paragangliomas (PGLs) are derived from the extra-adrenal sympathetic chromaffin (sympathetic PGLs) and/or parasympathetic tissue (non-chromaffin PGLs) of the head and neck (HNPGs) (Lenders et al. 2005). In 2004, the WHO defined malignant PCs/PGLs in the presence of metastatic disease without considering locally recurrent or invasive tumours (Ayala-Ramirez et al. 2011). Based on this classification, PCs exhibit a 15–20% 10-year probability of recurrence with a 20% malignancy rate (Ayala-Ramirez et al. 2011). Overall, metastatic PCs/PGLs are rare with an incidence of less than 1/1,000,000 population per year, with sympathetic PGLs being more common than PCs (60% vs 25%, respectively). However, no current treatment for malignant PCs/PGLs has been shown to be particularly effective (Lenders et al. 2014). Better understanding of the molecular pathways for the PCs/PGLs pathogenesis has led to the development of potential new therapeutic agents. Data from clinical trials using MTT are summarised in Table 1.

Transcriptomic studies have identified two main activated molecular pathways leading to the development of these tumours: the hypoxic (cluster-1 genes), mainly involving the succinate dehydrogenase (SDHx) and the Von Hippel-Lindau (VHL) genes, and the mTOR (cluster-2 genes) pathways, mainly involving rearranged during transfection (RET) and neurofibromin 1 (NFL) genes (Dahia et al. 2005, 2014, Favier et al. 2015). DNA-methylation profiling studies have uncovered a hyper-methyllator phenotype in the SDHx gene-related tumours (comprising SDHA/B/C/D and SDHAF2 genes) and showed that succinate acts as an onco-metabolite, inhibiting 2-oxoglutarate-dependent dioxygenases. ‘Omic’ data have also identified new therapeutic targets (Favier et al. 2015).

**Current treatment based on clinical trials**

Current guidelines suggest that the optimal initial treatment for metastatic PCs/PGLs should be surgical,
including total resection of the tumour, locoregional lymph nodes and distant metastases (Zografos et al. 2009, Lenders et al. 2014). For unresectable disease, combination chemotherapy with cyclophosphamide, vincristine and dacarbazine (CVD) has been associated with short-term symptomatic and tumour responses of vincristine and dacarbazine (CVD) has been associated with combination chemotherapy with cyclophosphamide, vincristine, and dacarbazine (CVD). Nölting & Grossman 2012, Zhang et al. 2012). Overexpression of key mTORC complex (mTORC) signalling mediators (Nölting & Grossman 2012, Zhang et al. 2012) is necessary for activation of the mTOR kinase. PRAS40 is another component and substrate of mTORC1 whose phosphorylation by mTORC1 results in its dissociation from mTORC1 inhibiting mTORC1 activity. Growth-factor-stimulated PI3K signalling also activates mTORC2, which regulates actin cytoskeletal organisation, ion transport and growth. mTORC2 consists of the mTOR catalytic subunit and at least five accessory proteins. Rictor, mSin1, and Protor are unique subunits of mTORC2. Everolimus is known to inhibit mTORC1, but increase Akt signalling. (B) MAPK pathway: signalling via the RAS-RAF-MEK-ERK cascade leads to phosphorylation of many substrates that can have multiple cellular effects. RAS-GTP activates RAF that activates the mTORC1. This was attributed to the selective inhibition of HIF-1 without any effect on HIF2, or to the compensatory activation of the RAS/RAF/ERK pathway (Semenza 2007, Druce et al. 2009, Nölting et al. 2012). In another study, all patients treated with everolimus experienced rapid disease progression (Druce et al. 2009). This was attributed to the selective inhibition of HIF-1 without any effect on HIF2, or to the compensatory activation of the RAS/RAF/ERK pathway (Semenza 2007, Druce et al. 2009, Nölting et al. 2012). In a subsequent phase II study using everolimus as monotherapy, five of seven patients with metastatic PC/PGLs achieved stable disease but with a progression-free survival (PFS) of only 3.8 months (95% CI: 0.5–7.0); four patients demonstrated tumour shrinkage (Oh et al. 2012). However, recent experimental studies with AZD8055, an ATP-competitive dual mTORC1/2 inhibitor, in metastatic mouse PC cell lines and in human primary cell models of patients with PC/PGLs, showed a significant decrease in tumour load with a 50% reduction in the number of malignant cells (Giubellino et al. 2013a). A recent in vivo study in mice xenografts confirmed these

Figure 1

(A) PI3K/Akt/mTOR pathway: The IGFR1 activates tyrosine kinase receptor through the lipid kinase PI3K/Akt pathway. PI3K activation phosphorylates and activates Akt that inhibits TSC1/TSC2 suppressor complex, which in turn activates mTORC1. PTEN is a major negative regulator of A activation. Akt-mediated phosphorylation of TSC1-TSC2 leads to the activation of the Rheb-GTPase- that activates the mTORC1. The mTOR signalling pathway serves as the central regulator of cell metabolism and proliferation. mLST8 is a common subunit of both mTORC1 and mTORC2, and is necessary for activation of the mTOR kinase. PRAS40 is another component and substrate of mTORC1 whose phosphorylation by mTORC1 results in its dissociation from mTORC1 inhibiting mTORC1 activity. Growth-factor-stimulated PI3K signalling also activates mTORC2, which regulates actin cytoskeletal organisation, ion transport and growth. mTORC2 consists of the mTOR catalytic subunit and at least five accessory proteins. Rictor, mSin1, and Protor are unique subunits of mTORC2. Everolimus is known to inhibit mTORC1, but increase Akt signalling. (B) MAPK pathway: signalling via the RAS-RAF-MEK-ERK cascade leads to phosphorylation of many substrates that can have multiple cellular effects. RAS-GTP activates RAF that activates the mTORC1. This was attributed to the selective inhibition of HIF-1 without any effect on HIF2, or to the compensatory activation of the RAS/RAF/ERK pathway (Semenza 2007, Druce et al. 2009, Nölting et al. 2012). In another study, all patients treated with everolimus experienced rapid disease progression (Druce et al. 2009). This was attributed to the selective inhibition of HIF-1 without any effect on HIF2, or to the compensatory activation of the RAS/RAF/ERK pathway (Semenza 2007, Druce et al. 2009, Nölting et al. 2012). In a subsequent phase II study using everolimus as monotherapy, five of seven patients with metastatic PC/PGLs achieved stable disease but with a progression-free survival (PFS) of only 3.8 months (95% CI: 0.5–7.0); four patients demonstrated tumour shrinkage (Oh et al. 2012). However, recent experimental studies with AZD8055, an ATP-competitive dual mTORC1/2 inhibitor, in metastatic mouse PC cell lines and in human primary cell models of patients with PC/PGLs, showed a significant decrease in tumour load with a 50% reduction in the number of malignant cells (Giubellino et al. 2013a). A recent in vivo study in mice xenografts confirmed these

Molecular targeted treatment for PCs/PGLs

mTOR inhibitors PCs/PGLs exhibit a significant overexpression of key mTOR complex (mTORC) signalling mediators (Nölting & Grossman 2012, Zhang et al. 2015) (Fig. 1A and B). Everolimus, a compound inhibiting mTOR and thus the PI3K/Akt/mTOR pathway, has been shown in vitro to inhibit the proliferation of human neuroblastoma cells, which are closely related to PCs/PGLs cluster-2-related tumours (Kiessling et al. 2016). Everolimus has previously been evaluated in malignant PCs but only in small series (Semenza 2007, Druce et al. 2009, Nölting et al. 2012). In another study, all patients treated with everolimus experienced rapid disease progression (Druce et al. 2009). This was attributed to the selective inhibition of HIF-1 without any effect on HIF2, or to the compensatory activation of the RAS/RAF/ERK pathway (Semenza 2007, Druce et al. 2009, Nölting et al. 2012). In a subsequent phase II study using everolimus as monotherapy, five of seven patients with metastatic PC/PGLs achieved stable disease but with a progression-free survival (PFS) of only 3.8 months (95% CI: 0.5–7.0); four patients demonstrated tumour shrinkage (Oh et al. 2012). However, recent experimental studies with AZD8055, an ATP-competitive dual mTORC1/2 inhibitor, in metastatic mouse PC cell lines and in human primary cell models of patients with PC/PGLs, showed a significant decrease in tumour load with a 50% reduction in the number of malignant cells (Giubellino et al. 2013a). A recent in vivo study in mice xenografts confirmed these
Table 1 Molecular-targeted agents used in different protocols of clinical trials completed or ongoing in patients with phaeochromocytomas (PCs) and paragangliomas (PGLs).

<table>
<thead>
<tr>
<th>Protocol IDs in ClinicalTrials.gov/Reference</th>
<th>Tested drug in the Test drug in the trial arme</th>
<th>Patients</th>
<th>Type</th>
<th>Status</th>
<th>Phase</th>
<th>Number of patients</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kulke et al. 2006</td>
<td>Thalidomide (VEGF-R, bFGF inhibitor) and Temozolomide (Alkylating chemotherapy)</td>
<td>Metastatic PCs/PGLs</td>
<td>Treatment Completed</td>
<td>II</td>
<td>3</td>
<td>1 had PR</td>
<td></td>
</tr>
<tr>
<td>NCT00468656, USA, 2007</td>
<td>Yttrium (Y) 90 octreotide acetate Everolimus (mTORC1 inhibitor) and vatalanib (EGFR-1 inhibitor)</td>
<td>Metastatic NETs including PCs/PGLs</td>
<td>Treatment Completed</td>
<td>II</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>NCT00655565, USA, 2008</td>
<td>Everolimus (mTORC1 inhibitor) and vatalanib (EGFR-1 inhibitor)</td>
<td>Metastatic and recurrent PCs</td>
<td>Treatment Active</td>
<td>I</td>
<td>ND</td>
<td>Not yet completed</td>
<td></td>
</tr>
<tr>
<td>Gonias et al. 2009</td>
<td>131I-MIBG</td>
<td>Metastatic PCs/PGLs</td>
<td>Treatment Completed</td>
<td>50</td>
<td>22% had CR 35% had PR (25% developed PD within 1 year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT00874614, USA, 2009</td>
<td>131I-MIBG</td>
<td>Metastatic NETs including PCs/PGLs</td>
<td>Treatment Active</td>
<td>II</td>
<td>ND</td>
<td>Not yet completed</td>
<td></td>
</tr>
<tr>
<td>NCT 00655655, USA, 2008</td>
<td>131I-MIBG</td>
<td>Metastatic NETs including PCs/PGLs</td>
<td>Treatment Active</td>
<td>II</td>
<td>ND</td>
<td>Not yet completed</td>
<td></td>
</tr>
<tr>
<td>NCT01155258, USA, 2010</td>
<td>Sunitinib (VEGF-R, PDGF-R inhibitor)</td>
<td>Recurrent or malignant PCs/PGLs</td>
<td>Treatment Completed</td>
<td>I</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>NCT01163383, USA, 2010</td>
<td>Temsirolimus (mTORC1 inhibitor) + vinorelbine ditartrate (anti-mitotic chemotherapy)</td>
<td>Metastatic PCs/PGLs</td>
<td>Treatment Active</td>
<td>II</td>
<td>Not yet completed</td>
<td>Not yet completed</td>
<td></td>
</tr>
<tr>
<td>NCT01371201, France, 2011 (FIRST-MAPP)</td>
<td>Sunitinib (VEGF-R, PDGF-R inhibitor)</td>
<td>Metastatic NETs including PCs/PGLs</td>
<td>Treatment Active</td>
<td>II</td>
<td>Not yet completed</td>
<td>Not yet completed</td>
<td></td>
</tr>
<tr>
<td>NCT01396408, Canada, 2011</td>
<td>Sunitinib (VEGF-R, PDGF-R inhibitor) vs Temsirolimus (mTORC1 inhibitor)</td>
<td>Unresected, advanced or metastatic rare tumours including PCs/PGLs</td>
<td>Treatment Active</td>
<td>II</td>
<td>Not yet completed</td>
<td>Not yet completed</td>
<td></td>
</tr>
<tr>
<td>Oh et al. 2012</td>
<td>Pazopanib hydrochloride (multi-TKI)</td>
<td>Advanced or progressive malignant PCs/PGLs</td>
<td>Treatment Completed</td>
<td>II</td>
<td>7</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>NCT0137753, USA, 2011</td>
<td>131I-MIBG</td>
<td>Malignant PCs/PGLs</td>
<td>Treatment Active</td>
<td>Expanded access</td>
<td>Not yet completed</td>
<td>Not yet completed</td>
<td></td>
</tr>
<tr>
<td>NCT0183818, USA, 2013</td>
<td>Everolimus (mTORC1 inhibitor)</td>
<td>Progressive PCs/PGLs</td>
<td>Treatment Completed</td>
<td>II</td>
<td>7</td>
<td>5 had SD had PD, PFS=3.8 months</td>
<td></td>
</tr>
<tr>
<td>NCT01967576, USA, 2013</td>
<td>Pazopanib hydrochloride (c-MET, VEGF-R)</td>
<td>Advanced PCs/PGLs</td>
<td>Treatment Completed</td>
<td>II</td>
<td>2</td>
<td>1 had SD for &gt;12 months</td>
<td></td>
</tr>
<tr>
<td>NCT 01340794, USA, 2011</td>
<td>Cabozantinib (TKI, c-MET, VEGF-R)</td>
<td>Advanced PCs/PGLs</td>
<td>Treatment Active</td>
<td>Expanded access</td>
<td>Not yet completed</td>
<td>Not yet completed</td>
<td></td>
</tr>
<tr>
<td>Oh et al. 2012</td>
<td>Fostamatinib Disodium (TKI)</td>
<td>Advanced PCs/PGLs</td>
<td>Treatment Completed</td>
<td>Pilot</td>
<td>Not yet completed</td>
<td>Not yet completed</td>
<td></td>
</tr>
<tr>
<td>NCT0183818, USA, 2013</td>
<td>131I-MIBG</td>
<td>Recurrent or primary unresectable PCs/PGLs</td>
<td>Treatment Active</td>
<td>Expanded access</td>
<td>Not yet completed</td>
<td>Not yet completed</td>
<td></td>
</tr>
</tbody>
</table>

VEGF-R, vascular endothelial growth factor-receptor; bFGF, b fibroblast growth factor; NET, neuroendocrine tumours; PC, phaeochromocytomas; PGLs, paragangliomas; ND, no data; mTOR, mammalian target of rapamycin; EGFR-1, endothelial growth factor receptor-1; MIBG, meta-iodobenzylguanidine; PR, partial response; CR, complete response; PD, progression disease; PDGF-R, platelet derived growth factor; TKI, tyrosine kinase; SD, stable disease; PFS, progression-free survival.
results, showing strong anti-tumour activity of the dual mTORC1/mTORC2 inhibitor PP242 (Zhang et al. 2015). This was due to the dual effect on both complexes TORC1/ TORC2 inhibiting the compensatory stimulation of the RAS/RAF/ERK pathway which is observed when a single mTORC1 inhibitor such as everolimus is used.

**Anti-angiogenic molecules** PCs are highly vascular, and aberrant angiogenesis has been associated with a malignant phenotype, especially in patients carrying SDHB gene mutations (Takekoshi et al. 2004). PC tumours express higher levels of VEGF and its receptors (VEGF-R), suggesting they may play an important role in their pathogenesis (Takekoshi et al. 2004). Interestingly, using antibodies against VEGF it was possible to reduce angiogenesis and tumour proliferation in a xenograft mouse model of PCs (Zielke et al. 2002). These findings have led to the evaluation of several angiogenesis inhibitors as therapeutic agents in malignant PGs/PGLs.

Thalidomide is an anti-angiogenic agent specifically targeting VEGF and the basic fibroblast growth factor (FGF) receptors. Only one phase II trial has been conducted, but due to the limited number of patients studied (n=3) it is impossible to draw definite conclusions regarding the efficacy of thalidomide in PC patients (Kulke et al. 2006).

Sunitinib, a potent oral multi-TK inhibitor with anti-angiogenic and anti-tumour activity, has demonstrated potential benefit in metastatic SDH- or VHL-mutated PCs (Jimenez et al. 2009, Saito et al. 2012). In cell lines, sunitinib decreased cell proliferation in vitro by targeting cell cycle, DNA metabolism and cell organisation genes (Cassol et al. 2014). In 17 patients with progressive metastatic PCs/PGLs, sunitinib was associated with a significant reduction in tumour size, a 30% decrease in 18Fluoro-deoxyglucose (FDG)-PET/CT uptake, and a median overall survival of 26.7 months with a PFS of 4.1 months (95% CI: 1.4–11.0) (Ayala-Ramirez et al. 2012a). Most patients who experienced a clinical benefit were carriers of the SDHB mutation (Hahn et al. 2009, Ayala-Ramirez et al. 2012a). In VHL patients with metastatic PCs/PGLs, treatment with sunitinib for 6 months was associated with normalisation of patients’ performance status and blood pressure, significant tumour shrinkage and reduction of plasma noradrenaline and chromogranin A levels (Jimenez et al. 2009). An ongoing phase II study (FIRST-MAPP) aims to determine the efficacy of sunitinib on PFS at 12 months in progressive malignant PCs/PGLs.

Vandetanib, which inhibits VEGF-R2, VEGF-R3 and VEGF-R1 and, at higher concentrations, EGF-R, has demonstrated promising results in both in vitro and in vivo studies (Brave et al. 2011); it is known to be active in medullary thyroid carcinomas harbouring RET mutations (Vitagliano et al. 2011). The coexistence of medullary thyroid carcinoma and PCs in MEN2 syndromes suggests that vandetanib could also be a good candidate drug in PCs/PGLs.

**Hypoxic-inducible factor inhibitors** Although no data have been reported in human malignant PCs with these agents, hypoxic-inducible factor (HIF) inhibitors have shown marked anti-tumour activity in various human tumour xenografts in mice (Semenza 2007). Under normoxic conditions, site-specific hydroxylation of HIF-1α allows recognition by the VHL protein resulting in proteasomal degradation. Under hypoxic conditions, hydroxylation of the HIF-1α subunit is suppressed, leading to increased levels of HIF that regulate the expression of many target genes. (Jochmanová et al. 2013, Yang et al. 2015).

Recently, the selective HIF-2 antagonist, PT2399, has been shown to induce tumour regression in mouse models of VHL-defective clear cell renal carcinoma characterised by inactivation of the VHL gene and subsequent activation of HIF-2 (Cho et al. 2016). PT2399 had greater activity than sunitinib, was active in sunitinib-progressing tumours, and was better tolerated (Chen et al. 2016). These data suggest that this drug may be useful in PCs/PGLs associated with HIF-2a mutations.

**Heat shock protein-90 (HSP90)** This multi-chaperone ATP-dependent complex is responsible for protein folding and plays an important role in the stability and function of a number of oncoproteins and telomerase (Banerji 2009). Overexpression of HSP90 has been observed in malignant PCs, and it has been identified as a promising therapeutic target (Banerji 2009). In a xenograft mouse model, the use of two different HSP90 inhibitors, benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin (17-AAG), and the second-generation ganetespib, demonstrated significant inhibition of proliferation and migration in PC cell lines and induced degradation of HSP90. In these metastatic models of PCs, both inhibitors achieved reduction of tumour burden and reduced cell survival (Giubellino et al. 2013b). HSP90 may thus represent a potential therapeutic target for metastatic PCs/PGLs, although the relatively non-specific mode of action might limit its use.
**HER2/neu inhibitors** HER2 is a tyrosine kinase receptor involved in the cell growth and differentiation and it leads to the synthesis of HIF when activated. HER2 is overexpressed in malignant PCs and is associated with tumour metastases and resistance to treatment; however, no trials have been conducted to date using HER2/neu inhibitors (Tavanger et al. 2010, Mohammed et al. 2014).

**Other agents** Carboxypeptidase E (CPE) is a prehormone-processing enzyme expressed in different types of cancer including NETs, mainly of the lung and pituitary (Murthy et al. 2010). Extremely high CPE mRNA copy numbers of the N-terminal splice isoform variant were found in various human metastatic tumour cell lines, including metastatic PCs. Interestingly, this high copy number in tumours was shown to predict tumour recurrence and/or metastatic disease, suggesting that CPE could be a potential therapeutic target in metastatic PCs.

NVP-AEW451 acts as IGF-1 receptor antagonist that significantly reduced mouse PC cell proliferation and tumour cell viability, albeit at relatively high doses. However, at suboptimal doses this agent led to a compensatory upregulation of ERK and mTORC1 signals whereas PI3K/AKT inhibition remained stable (Nölting et al. 2012). More recently, LB1, a small molecule inhibitor of serine/threonine protein phosphatase 2A, inhibited mouse PC cells in vitro and in vivo in a mouse model of metastatic PCs, either alone or in combination with temozolomide (Martiniova et al. 2011).

Temozolomide, an oral derivative of dacarbazine, was studied as monotherapy in 15 patients with metastatic PCs/PGLs. There were five partial responses (33%), seven (47%) patients experienced stable and three (20%) developed progressive disease after a median follow-up of 35 months. Partial responses were observed only in patients with SDHB mutations. The silencing of O(6)-methylguanine-DNA methyltransferase (MGMT) expression as a consequence of MGMT promoter hypermethylation in SDHB-mutated tumours may explain this finding (Hadox et al. 2014).

Another promising therapeutic agent could be the use of lipophilic statins (simvastatin and fluvastatin) either alone (Fliedner et al. 2014) or in association with 13-cis-retinoic acid (Nölting et al. 2014). Statins can induce apoptosis of PC cells through inhibition of MAPK-1 and -3 phosphorylation in aggressive mouse PC cells in vitro (Fliedner et al. 2014, Nölting et al. 2014).

In vitro studies in tissues as well as in cell cultures have revealed the expression of somatostatin receptors (SSTRs) (particularly subtype 2A and 3) in PCs/PGLs and have suggested that by targeting SSTRs it might be able to control tumour growth and secretion (Ziegler et al. 2009, Elston et al. 2015). However, these results have not been widely confirmed, as only some case series have shown symptom and tumour control in a subset of patients (van Hulsteijn et al. 2013, Elshafei et al. 2014). More promising results with radioisotopes bound to synthetic somatostatin analogues (SSAs) in patients with high tumoural uptake have been published, especially significant in the light of the impressive results of peptide receptor radiotherapy (PRRT) reported in the NETTER-1 study in patients with midgut neuroendocrine tumours (Strosberg et al. 2017). To date, PRRT using ^{90}Yttrium or ^{177}Lutetium-labelled SSAs has been evaluated in only a limited number of PCs/PGLs. In a total of 25 patients from three different clinical trials including patients with progressive or metastatic PCs/PGLs, four patients showed a partial response and 14 had stable disease, suggesting that PRRTs could be an effective therapeutic option for these tumours (van Essen et al. 2006, Zovato et al. 2012, Puranik et al. 2015). Larger studies including hereditary and non-hereditary PCs/PGLs are needed to identify which PCs/PGLs can be best treated using this therapy, and whether PRRT should be used alone or with other treatment modalities.

Radionuclide therapy with ^{131}I-metaiodobenzylguanidine (MIBG) has also been used either as a single or sequential cumulative doses administered in patients with significant tumour burden and adequate ^{123}I-MIBG uptake on diagnostic imaging (Mukherjee et al. 2001, Kalsats et al. 2005b). A recent meta-analysis of the efficacy of ^{131}I-MIBG-therapy in malignant PCs/PGs showed that disease control and partial hormonal responses were obtained in 50 and 40% of patients, respectively (van Hulsteijn et al. 2014). In a multicentre registry, partial response or stability of the disease according to the RECIST criteria was achieved in 85% of patients, with minor side effects in approximately 90% of patients (Yoshinaga et al. 2014). However, there may also be long-term sequelae such as myelodysplastic disorders and haematological malignancies (Sze et al. 2013). Nevertheless, the prognosis for malignant PCs/PGLs still remains relatively poor, highlighting the need for the development of new personalised therapeutic strategies.

In summary, for metastatic PCs/PGLs that do not respond or cannot be treated with the current established therapies, radionuclide therapy with a labelled SSTR-active analogue or ^{131}I-MIBG should be considered, depending on the diagnostic uptake. At present, it is not possible to differentiate between these two possible therapies. MTT
Adrenocortical carcinoma

Introduction

Adrenocortical carcinoma (ACC) is a rare tumour with an estimated incidence of 0.7 new cases/1,000,000 per year (Assie et al. 2014). The genomic landscape of ACCs has revealed that it is a biologically and genetically heterogeneous malignancy with transcriptome clusters associated with distinct drugable clinical behaviour (Assie et al. 2014). Various molecular agents used in recent years in clinical trials as well as in vitro studies for treating patients with ACC have been already well-described in a recent review (Creemers et al. 2016). Therefore, here we summarise most of these data as well the newer studies published since this review in Tables 2 and 3.

Current treatment based on clinical trials

Currently ‘R0’, complete surgical resection, is the gold standard treatment for non-metastatic ACC or following local recurrence (Berruti et al. 2012b). Mitotane is the only FDA-approved drug for locally advanced inoperable and metastatic disease displaying single-agent activity of 10–30% tumour response rates based on its adrenolytic action, albeit with a high toxicity profile (Veytsman et al. 2009). The only prospective phase III randomised clinical trial, FIRM-ACT, showed that in advanced ACCs mitotane combined with etoposide, doxorubicin and cisplatin (EDPM) provided some additional clinical benefit compared to mitotane plus streptozocin alone, but was associated with more serious adverse events (Fassnacht et al. 2012). A further prospective study evaluating mitotane vs. placebo in high-risk for recurrence patients, ADIUVO, is currently under way (Terzolo et al. 2007) (https://www.epiclin.it/adiuvo).

Molecular profile

The main signalling pathways involved in ACC tumourigenesis include the PI3K/Akt/mTOR cascade and the RAS-MAPK pathway, both activated by the IGF-1R when occupied by IGF2 (Pollak 2008). IGF2 mRNA and protein are overexpressed in more than 90% of ACCs (Ribeiro et al. 2012). Genetic studies have identified alterations in the FGF-R cascade (Laurell et al. 2009), and overexpression of VEGF-R, PDGF-R, EGFR, and the FGF-R signalling pathways (Xu et al. 2011, Wang et al. 2012). Preclinical studies support the idea that mTOR inhibitors can upregulate Akt phosphorylation in ACCs (Liu et al. 2009, De Martino et al. 2014).

Other key molecular events contributing to the formation of ACC are tumour protein S3 (TP53) inactivating mutations (Libè et al. 2007) and constitutive activation of the Wnt/β-catenin signalling pathway via activation of the β-catenin gene (CTTNB1) (Tissier et al. 2005). The increased occurrence of adrenocortical tumours in Li-Fraumeni and Beckwith-Wiedemann syndromes, as well as in the Carney complex, has highlighted the roles of susceptibility genes: TP53, IGF2, and protein kinase cAMP-dependent type I regulatory subunit alpha (PRKAR1A) (de Joussineau et al. 2012), respectively.

Alterations in the nuclear transcription steroidogenic factor 1 (SF-1) are also involved (Val et al. 2003, Duregon et al. 2013). SF-1 induces proliferation of ACC cell lines and tumour growth in vivo (Doghman et al. 2009) and its increased expression has been associated with a worse prognosis (Sbiera et al. 2010). SF-1 inhibitors in ACC cell lines inhibited cell proliferation associated with SF-1 overexpression along with steroid hormone oversecretion and CYP21 and CYP17 mRNA expression (Doghman et al. 2010).

Interferon-β (INF-β) may exert an inhibitory effect in vitro on ACC cell lines and primary cultures of human ACC (van Koetsveld et al. 2013), and increases sensitivity of ACC cells to mitotane (van Koetsveld et al. 2013).

Topoisomerase-a2 (TOP2A), a gene consistently overexpressed in ACC, is involved in cellular invasion as it regulates anchorage-independent growth and invasion. Several TOP2A inhibitors have been screened for their anti-proliferative activity in ACC cells (Jain et al. 2013).

Molecular therapy

Different drugs targeting the molecular pathways described above have been tried in clinical trials phase I–III (Table 2) or in vivo and in vitro studies (Table 3). IGF-1R inhibitors including mTOR inhibitors and monoclonal anti-IGF-1R antibodies, as well as anti-angiogenic agents, have been used as monotherapy in
Table 2 Molecular-targeted agents used in clinical trials completed or ongoing in patients with adrenocortical carcinoma.

<table>
<thead>
<tr>
<th>Molecular-targeted treatments</th>
<th>Molecular pathway</th>
<th>Design of the study</th>
<th>N (patients)</th>
<th>Response</th>
<th>Toxicities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1 blocker</td>
<td>Linsitinib (OSI-906) inhibitor IGF-1R and insulin receptor</td>
<td>I 9</td>
<td>2 had PR and 7 had SD</td>
<td>Grade 1 (nausea, vomiting, fatigue, diarrhoea, hyperglycaemia)</td>
<td>Jones et al. 2015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Linsitinib (OSI-906) inhibitor IGF-1R and insulin receptor</td>
<td>III 139</td>
<td>Stopped prematurely due to failure to improve PFS or OS</td>
<td>Grade 1 (fatigue, nausea, hyperglycaemia)</td>
<td>Fassnacht et al. 2015</td>
<td></td>
</tr>
<tr>
<td>Everolimus</td>
<td>mTOR inhibitor</td>
<td>Case series 4 (2/4 of them were also on mitotane)</td>
<td>All patients had PD</td>
<td>Well tolerated</td>
<td>Fraenkel et al. 2013</td>
<td></td>
</tr>
<tr>
<td>Immunotherapy</td>
<td>Figitumumab Monoclonal Ab targeting IGF-1R</td>
<td>I 14</td>
<td>8 (57%) had SD</td>
<td>Grade 1 (hyperglycaemia, nausea, fatigue, anorexia)</td>
<td>Haluska et al. 2010</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nivolumab Monoclonal Ab PD-1</td>
<td>II ND</td>
<td>Recruiting</td>
<td>Recruiting</td>
<td>NCT02720484, USA, 2016</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pembrolizumab Monoclonal Ab IgG4</td>
<td>II ND</td>
<td>Recruiting</td>
<td>Recruiting</td>
<td>NCT02673333, USA, 2016</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cixutumumab Monoclonal Ab targeting IGF-1R</td>
<td>II ND</td>
<td>Recruiting</td>
<td>Recruiting</td>
<td>NCT01514526</td>
<td></td>
</tr>
<tr>
<td>Angiogenetic factors</td>
<td>Axitinib (AG-013736) Inhibitor of VEGF-R (TKIs)</td>
<td>II 13</td>
<td>Median PFS = 5.48 months, median OS = 13.7 months,</td>
<td>Most Grade 1,2</td>
<td>O’Sullivan et al. 2014</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sunitinib Multiple TKIs</td>
<td>II 35</td>
<td>5 had SD, median PFS = 2.8 months</td>
<td>Grade 1,2 (fatigue, hand-foot reactions, rash, mucostitis)</td>
<td>Kroiss et al. 2012</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Imatinib Inhibitor of PDGF-R</td>
<td>II 4</td>
<td>0/4 (No response to any patients)</td>
<td>Grade 1 (nausea, diarrhoea, fatigue) 1 patient had grade 4 (psychiatric adverse events)</td>
<td>Gross et al. 2006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dovitinib Inhibitor of FGF-R</td>
<td>II 17</td>
<td>1 had PR, 13 had PD, median PFS = 1.8 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gefitinib Inhibitor of EGF-R</td>
<td>II ND</td>
<td>no improvement in OS</td>
<td>ND</td>
<td>Jesús García-Donas et al. 2014</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Suramin (Polysulphonated naphthylurea) Combinations</td>
<td>II ND</td>
<td>ND</td>
<td>ND</td>
<td>NCT00215202, USA, 2005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sorafenib + paclitaxel Inhibitor of VEGF-R2, VEGF-R3, PDGF-R, RAF-1 + chemotherapy</td>
<td>II 25</td>
<td>Stopped prematurely due to failure to improve PFS or OS</td>
<td>Grade 2.3 (fatigue, hypophosphatemia)</td>
<td>Berruti et al. 2012a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Erlotinib + gemcitabine Inhibitor of EGFR-Inhibitor + nucleoside analogue (chemotherapy)</td>
<td>Case series 10</td>
<td>1 had PR, median PFS = 8 months</td>
<td>Grade 1 (rash, fatigue)</td>
<td>Quinkler et al. 2008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bevacizumab + capecitabine Anti-VEGF Ab + 5-FU analogue (chemotherapy)</td>
<td>I 10</td>
<td>All patients had PD</td>
<td>Grade 1 (hand-foot syndrome)</td>
<td>Wortmann et al. 2010</td>
<td></td>
</tr>
</tbody>
</table>
patients with advanced progressive ACCs, albeit without promising results. The most probable explanation for this finding is that the inhibition of IGF-R1 leads to the compensatory activation of the MEK/ERK pathway leading to sustained activation of mTOR (Xu et al. 2016). FGF-R4 overexpression and amplification (identified in 13% of paediatric and 30% of adult ACCs) was associated with a worse outcome, supporting the potential role of selective FGF-R inhibitors in ACC treatment (West et al. 2007, Brito et al. 2012).

Treatments combining different molecular targeting agents or a molecular targeting agent with classical chemotherapy have been also studied. Preclinical data suggest increased efficacy of sunitinib when combined with an ERK pathway inhibitor (Lin et al. 2012). However, the plasma levels of sunitinib might be reduced by mitotane-induced CYP3A4 activity, thereby attenuating its anti-tumour activity when used in combination (Kroiss et al. 2012). Co-inhibition of IGF-R1 and EGF-R has also shown promising results in ACC cell lines.

Based on the premise that many ACCs require substantial intracellular cholesterol as a substrate for steroidogenesis, drugs disrupting cholesterol uptake might have therapeutic potential. A selective inhibitor of acetyl-CoA acetyl-transferase 1 (ACAT1), which catalyses cholesterol ester formation from cholesterol and long-chain fatty acyl-CoA, has been investigated in a phase II study of patients with advanced ACC (Aung Naing et al. 2015).

Other molecules including inhibitors of polo-like kinase (PLK-1), such as BI-236 (Bussey et al. 2016), inhibitors of TOP2A such as aclarubicin (Jain et al. 2013) and IL-13-pseudomonas exotoxin (Liu-Chittenden et al. 2015), are attractive strategies in ACC. Three other compounds that have been investigated in preclinical ACC models are thiazolidinediones (TZDs), HSP90 inhibitors, and decitabine, a DNA methyl-transferase inhibitor, with all showing inhibition of ACC cell proliferation (Suh et al. 2010, Huang et al. 2014).

In clinical practice, the gold standard treatment for metastatic ACC is cytoreductive surgery along with mitotane in combination with chemotherapy (EDP) according to the FIRM-ACT study. For progressive disease, despite these treatments their outlook remains still grim, with little assistance as yet from monotherapy with MTTs. The combination of imatinib with dacarbazine and capcitabine, or cixutumumab and temsirilimus, has shown encouraging clinical responses, albeit with considerable adverse events. As yet, MTTs have had little impact on the therapy of this sinister disease.
### Table 3  Molecular-targeted agents used in *in vitro*/*in vivo* studies of adrenocortical carcinoma.

<table>
<thead>
<tr>
<th>Molecular-targeted treatments</th>
<th>Molecular pathway</th>
<th><em>in vitro/in vivo</em></th>
<th>Response</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IGF-1 blocker</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Everolimus</td>
<td>mTOR inhibitor</td>
<td><em>In vitro</em> ACC cell lines in childhood</td>
<td>Inhibition of cell proliferation and reduced tumour cell growth</td>
<td>Doghman <em>et al.</em> 2010</td>
</tr>
<tr>
<td><strong>Angiogenetic factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunitinib</td>
<td>Multiple TKIs</td>
<td><em>In vitro</em> (H295R and SW13 ACC cells lines)</td>
<td>Reduce cell proliferation by 20%</td>
<td>Lin <em>et al.</em> 2012</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>Anti-EGF-R</td>
<td><em>In vitro</em> (H295 and SW13 ACC cells lines)</td>
<td>Reduction of cell viability in ACC lines</td>
<td>Gagliano <em>et al.</em> 2015</td>
</tr>
<tr>
<td><strong>Combinations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorafenib + everolimus</td>
<td>VEGF-R2, VEGF-R3, PDGF-R and RAF-1 + mTOR inhibitors</td>
<td><em>In vitro</em> (SW13 cells lines)</td>
<td>Significant apoptosis</td>
<td>Berruti <em>et al.</em> 2012b</td>
</tr>
<tr>
<td>Sorafenib + everolimus</td>
<td>VEGF-R2, VEGF-R3, PDGF-R and RAF-1 + mTOR inhibitors</td>
<td><em>In vitro</em> (SW13 and H295R cells lines)</td>
<td>Inhibition of cell viability in SW13 cells</td>
<td>Marinello <em>et al.</em> 2012</td>
</tr>
<tr>
<td>Sirolimus + mitotane</td>
<td>mTORC1/C2 and adrenolytic treatment</td>
<td><em>In vitro</em> (H295 and SW13 cells lines)</td>
<td>Mitotane significantly inhibited cell proliferation. Sirolimus showed statistically significant additive effects</td>
<td>De Martino <em>et al.</em> 2016</td>
</tr>
<tr>
<td>Sunitinib + PD98059</td>
<td>TKIs + ERC inhibitor (Raf/MEK inhibitor)</td>
<td><em>In vitro</em> (H295R and SW13 ACC cells lines)</td>
<td>Decrease proliferation by 68% and 64% in H295R and in SW13 cells</td>
<td>Lin <em>et al.</em> 2012</td>
</tr>
<tr>
<td>Erlotinib + NVP-AEW541</td>
<td>EGF-R inhibitor + IGF-1R inhibitor</td>
<td><em>In vitro</em> (H295R and SW13 ACC cells lines)</td>
<td>Inhibition of cell viability and increase apoptosis.</td>
<td>Xu <em>et al.</em> 2016</td>
</tr>
<tr>
<td>IFN-b + mitotane</td>
<td>Interferon-b + adrenolytic chemotherapy</td>
<td><em>In vitro</em> (ACC mice xenografts)</td>
<td>Antitumor activity <em>in vivo</em></td>
<td>Van Koetsveld <em>et al.</em> 2013</td>
</tr>
<tr>
<td><strong>Inhibitors of steroidogenesis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-density lipoprotein (HDL) nanoparticles</td>
<td>SR-BI inhibitors</td>
<td><em>In vitro</em> (H295R and SW13 cells lines)</td>
<td>Enhanced the apoptosis induced by etoposide, cisplatin or mitotane</td>
<td>Subramanian <em>et al.</em> 2016</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkylphenol and isoquinolinone</td>
<td>SF-1 inhibitors</td>
<td><em>In vitro</em> (H295R and SW13 cells lines)</td>
<td>Inhibitory effect on both SF-1-positive and -negative cells</td>
<td>Doghman <em>et al.</em> 2010</td>
</tr>
<tr>
<td>Bi-2536</td>
<td>Inhibitors of PLK-1</td>
<td><em>In vitro</em> (H295R and SW13 cells lines)</td>
<td>70% loss of viability</td>
<td>Bussey <em>et al.</em> 2016</td>
</tr>
<tr>
<td>Aclarubicin</td>
<td>TOP 2A inhibitors</td>
<td><em>In vitro</em> (H295R and SW13 cells lines)</td>
<td>Decrease of the proliferation and tumour spheroid size (P &lt; 0.05)</td>
<td>Jain <em>et al.</em> 2013</td>
</tr>
</tbody>
</table>

IGF-1R, insulin-like growth factor 1 receptor; mTOR, mammalian target of rapamycin; ACC, adrenocortical cancer; TK1, tyrosine kinase inhibitor; VEGF-R, vascular endothelial growth factor receptor; EGF-R, epidermal growth factor receptor; FGF-R, fibroblast growth factor receptor; PDGF-R, platelet-derived growth factor receptor; ERK, extracellular signal-regulated kinases (MAP kinases), SR-BI, scavenger receptor class B type I; SF-1, steroidogenic factor 1; PLK1, Polo-like kinase 1TOP2A, topoisomerase (DNA) II Alpha.
Pituitary tumours

Introduction

Pituitary tumours are for the most part benign monoclonal adenomas presenting with excessive hormone secretion and/or tumour mass effects, albeit a small minority may become frankly malignant (Melmed et al. 2003). Although surgical resection remains the mainstay of therapy for macroadenomas causing compression of neurovascular structures, pharmacotherapy can play a crucial role in their treatment. Recent advances in the genetic and molecular analysis of pituitary tumours have provided new insights into the growth patterns and secretory function of these tumours, and have allowed for a more precise characterisation of individual lesions.

Molecular profile

The PI3K/Akt and MAPKs pathways are the two major signalling pathways responsible for regulating cell growth and proliferation, which are activated by growth factor receptors (McCubrey et al. 2007). The PI3K/Akt/mTOR pathway is also upregulated in pituitary tumours (Muşat et al. 2005, 2010, Cakir et al. 2009). Notch-3 and Jagged-1 have been implicated in the pathogenesis of human non-functioning pituitary adenomas, thereby providing a potential therapeutic target for the medical treatment of these tumours. Several elements of the Notch pathways have also been identified in the transcriptome of prolactinomas and multi-hormonal pituitary adenomas (Jiang et al. 2012). Hedgehog (Hh) signalling exerts differential effects on pituitary cell growth (with stimulatory effects on progenitor cells and inhibitory effects in differentiated cells), and is able to modulate hormone secretion and proliferation in pituitary tumours. Moreover, exogenous treatment with Hh proteins in cell cultures has been shown to increase hormone secretion from pituitary tumour cells, reflecting their role as hypophysiotropic factors that regulate pituitary hormone release in normal and tumoural tissue cells (Yavropoulou et al. 2015). More specific targeting treatment depends on the type of pituitary adenoma.

Approximately, 30–45% of pituitary tumours invade the cavernous or sphenoid sinus (Zada et al. 2011), and a significant number are considered as aggressive based on their resistance to conventional treatment or recurrence during the follow-up (Raverot et al. 2012) (Fig. 2). Some rare aggressive tumours that develop metastases are considered as carcinomas (Heaney et al. 2011, Batisse et al. 2013, Chatzellis et al. 2015) and cannot be controlled by any available treatment (McCormack et al. 2009, Heaney et al. 2011, Jouanneau et al. 2012, Raverot et al. 2012). Pituitary carcinomas are rare (0.2%), defined by the presence of systemic or cerebrospinal metastases (Kaltsas et al. 2005a, Heaney et al. 2011). The 2004 WHO classification considered all benign tumours as either typical adenomas or atypical adenomas tumours showing ‘borderline or uncertain behaviour’ (Keblad et al. 2007). The latter tumours exhibited invasive growth, high mitotic index, an elevated mitotic index, a Ki-67 labelling index (LI) >3% as well as extensive nuclear staining for p53 (Del Basso De Caro et al. 2016, Kim et al. 2016). In recent studies, pituitary tumour recurrence could be predicted by using mitoses, invasion, Ki-67 labelling index (>3%), or extensive p53 immunoreactivity (≥20%) (Del Basso De Caro et al. 2016, Kim et al. 2016). However, it should be noted that the revised WHO classification, due for publication in 2017, has rejected the ‘typical/atypical’ classification, and instead emphasises staining with Ki-67, and the degree of invasiveness as revealed by surgical and radiological findings; p53 staining should be reserved for special cases, whereas electron microscopy is rarely of value.

Current treatments for aggressive tumours

Because of the rarity of these tumours, data from randomised controlled studies comparing the efficacy and safety of

Figure 2

Post-contrast T1-sequence MRI showing an aggressive pituitary macroadenoma recurring after initial surgery.
the various therapeutic modalities are lacking. Patients with incompletely resected aggressive tumours may require further surgery and additional medical treatment with SSAs and/or cabergoline. Recently, dopamine agonist therapy in non-functioning pituitary adenomas with post-surgical remnants showed stabilisation or shrinkage of the tumour (Greenman et al. 2016). Conventional or targeted radiotherapy is worthy of consideration, whereas conventional systemic chemotherapy has been shown to have an unpredictable and generally minor response (Kaltsas et al. 2005b, Raverot et al. 2012).

Somatostatin receptor expression is found within normal pituitary cells including corticotrophs and lactotrophs, and binding of somatostatin to its receptor triggers a G-protein-mediated signal cascade that inhibits secretory function in these cells. The two long-acting SSAs in use today are octreotide and lanreotide. More recently, the somatostatin analogue pasireotide, showing increased binding affinity for SSTR-1, 3 and 5, was compared to octreotide and lanreotide, and has apparently shown superior efficacy in the biochemical control of patients with acromegaly (Colao et al. 2014). Pegvisomant, a pegylated analogue of human GH, is currently the only GH-receptor antagonist approved by the FDA available for the treatment of acromegaly. It directly competes for receptor binding with plasma GH resulting in decreased IGF-1 production (van der Lely et al. 2012).

Recent clinical trials have demonstrated the successful application of temozolomide in pituitary carcinomas and aggressive pituitary tumours, and this agent has shown to be efficient in controlling tumour progression and metastasis in approximately 50% of cases (McCormack et al. 2009, McCormack et al. 2011, Raverot et al. 2012). Despite these encouraging results with temozolomide showing long-term control in 40% of patients (Bush et al. 2010, Losa et al. 2010, Raverot et al. 2010), some tumours develop secondary resistance during the follow-up (McCormack et al. 2009, Raverot et al. 2010). The development of new therapeutic options is particularly necessary for pituitary carcinomas resistant to temozolomide (Jouanneau et al. 2012). Some case reports suggest the need to combine temozolomide with other chemotherapeutic agents such as capecitabine (Thearle et al. 2011) or with the new SSA pasireotide (Bode et al. 2010).

Molecular targeted treatment

A number of preclinical and clinical studies have suggested that new MTT may also be useful in controlling pituitary tumour growth. The anti-proliferative effect of an mTOR inhibitor on different pituitary cell lines or primary cultures has been demonstrated in vitro (Cerovac et al. 2010). In vitro data have also shown an effect of everolimus on cell viability in cell cultures from non-functioning pituitary adenomas (Zatelli et al. 2010). Furthermore, a mutation of the mTOR pathway, STK1, was found in a woman with an adrenocorticotrophic-secreting pituitary carcinoma refractory to surgical resection, radiation, and chemotherapy with capecitabine and temozolomide. After the combination of radiation and everolimus, significant clinical improvement and radiological stability was obtained, although the patient died of metastatic disease (Vlotides et al. 2008).

There is also some evidence supporting the role of the EGF-R pathway in pituitary proliferation (Vlotides et al. 2008, Cooper et al. 2011), and the potential use of TKIs (Fukuoka et al. 2011) for targeted therapy in pituitary tumours. Anecdotal cases have shown control of tumour growth by the administration of bevacizumab, an anti-VEGF agent, in a single patient (Ortiz et al. 2012), and in another case 26-month disease control was observed (Ortiz et al. 2012). The previously highlighted importance of EGF and its receptor EGF-R has also prompted research concerning the use of TKIs, especially the EGFR inhibitor gefitinib, as a targeted medical therapy for ACTH adenomas, demonstrating promising in vitro results (Fukuoka et al. 2011).

Recently, an exhaustive exome-wide screening has led to the identification of somatic mutations in the ubiquitin-specific protease 8 (USP8) in ACTH-producing adenomas (Reincke et al. 2015). This gene codes for a protein with deubiquitinase (DUB) activity that inhibits the lysosomal degradation of EGF-R (Mizuno et al. 2005). Mutated USP8 leads to higher DUB activity than the wild type, therefore increasing EGF-R stability and enhancing EGF-R-induced pro-opiomelacortin (POMC) transcription and ACTH secretion. The absence of USP8 mutations in other types of pituitary tumours, including silent ACTH-producing adenomas, suggests that these alterations are specific traits of secretory corticotroph adenomas causing Cushing’s disease (Reincke et al. 2015). The high prevalence of mutations in USP8 in patients with Cushing’s disease, but not in any other pituitary adenomas, provides a plausible explanation for the dependence of corticotrophinomas on EGF-R signalling and suggests that the mutational status of USP8 can be used to stratify the patients for targeted therapies against EGFR (Fukuoka et al. 2011, Ma et al. 2015). In vitro studies showed that targeting EGF-R to mouse corticotroph cell nuclei resulted in higher POMC expression and ACTH.
secretion, both of which were inhibited by gefitinib (Fukuoka et al. 2011).

The cyclin-dependent kinase (CDK) inhibitor p16 is downregulated in pituitary tumours, probably by methylation of its promoter, leading to a loss of suppression of CDK4 and CDK6 and progression through the checkpoint inhibition into the cell-cycle S phase (Farrell & Clayton 2003). Recently, CDK4/6 antagonists have entered into clinical practice, with palbociclib showing potential in the treatment of breast cancer, causing either cellular quiescence or senescence (Finn et al. 2016). It will be of interest to see whether such treatment will be of value in the therapy of aggressive pituitary adenomas or carcinomas. However, it should be noted that corticotroph tumours are more dependent on alterations in p27, cyclin E and CDK2; therefore, these tumours may be less likely to respond (Dworakowska & Grossman 2012).

Although PRRT directed against SSTR2 and SSTR5 subtypes using either 90Ytrium and most recently 177Lutetium could be used for aggressive or recurrent pituitary tumours expressing SSTRs, this modality has not been significantly used in pituitary carcinomas. There are only few case reports in the literature demonstrating the use of PRRT in atypical or aggressive pituitary adenomas or carcinomas. 177Lutetium DOTATOC improved local complications for more than 8 years after ineffective surgery and gamma-knife therapy in a patient with a non-functioning pituitary macroadenoma (Komor et al. 2014). In another case, a patient with an aggressive, giant GH-secreting tumour refractory to all treatments developed a partial biochemical remission and reduction in tumour mass using 90Y-DOTATATE (Waligorska-Stachura et al. 2016). In a further case series including one patient with a pituitary carcinoma and two with atypical adenomas, results were inconsistent as stable disease was achieved in the sole patient with the more slowly progressive tumour (Maclean et al. 2014). Furthermore, a patient with a giant prolactinoma, refractory to medical treatment, showed substantial tumour shrinkage after four cycles of 111Indium-DTPA-octreotide (Baldari et al. 2012), but this radiobiological therapy is far from ideal.

Overall, for malignant pituitary tumours there is limited evidence for the role of MTT, although TKIs such as gefitinib and mTOR inhibitors such as everolimus have demonstrated promising results in vitro and in vivo in animals. However, as these tumours exhibit SSTRs, treatment with PRRT appears an attractive option, probably best in the context of a therapeutic trial. Nevertheless, in desperate situations there may be a place for attempted therapy with either gefitinib or everolimus. The potential role of chemosensitisation with temozolomide to enhance the therapeutic response could also be explored in the future.

**Parathyroid carcinoma**

**Introduction**

Most cases of primary hyperparathyroidism are sporadic, but may occur as a part of hyperparathyroidism-jaw tumour syndrome (HJTS), or multiple endocrine neoplasia type-1 or 2A syndromes (McClenaghan et al. 2015). Histologically, primary hyperparathyroidism is caused by a single adenoma in 80–85% of cases, by hyperplasia or multiple adenomas in 15–20% of cases, and by a carcinoma in 0.5–5% of cases (Givi & Shah 2010, Mohebati et al. 2012) (Fig. 3). Differentiation of parathyroid carcinoma from parathyroid adenoma, especially atypical adenoma, can be very difficult in some cases (Mohebati et al. 2012).

**Molecular profile**

Gene expression profiling of parathyroid adenomas and carcinomas may provide an explanation for the formation of parathyroid carcinomas and aid in the differential diagnosis of these lesions (Arvai et al. 2012). To date, mutations of one hyperparathyroidism gene (HRPT2, also known as CDC73) has been established as the underlying genetic mechanism in parathyroid
carcinoma tumourigenesis (Carpten et al. 2002). Inactivating HRPT2 mutations are frequently revealed in germ line cells of these patients, and are associated with a syndromic form of the disease known as the HJTS (Juhlin et al. 2006).

Parafibromin, the protein product of HRPT2, has also been linked to the Wingless type (Wnt) pathway through its nuclear association with β-catenin (Mosimann et al. 2006). In the absence of Wnt signalling, β-catenin is phosphorylated and subsequently degraded by a protein complex consisting of axin, adenomatous polyposis coli (APC) and glycogen synthase kinase 3-β (GSK3-β) (Van Noort et al. 2002), which have been associated with parathyroid carcinoma development. In vitro studies have shown a loss of APC and GSK3-β immunoreactivity in sporadic parathyroid carcinomas without an increase in β-catenin or cyclin D1 levels (Juhlin et al. 2009). The normal function of GSK3-β would theoretically be in agreement with a tumour suppressor function; however, inactivating alterations of the GSK3-β gene have not been reported in human cancers (Karim et al. 2004).

Current treatment

The treatment of choice for parathyroid carcinoma is surgery. Therapeutic options for non-resectable and metastatic parathyroid carcinoma are limited. The primary aim is to control hypercalcaemia, and although mitramycin, plicamycin, gallium nitrate, bisphosphonates, calcitonin and glucocorticoids have been used, the calcimimetic cinacalcet is the most effective (Wei & Harari 2012). Locoregional treatments including ethanol ablation, radiofrequency ablation and trans-arterial embolisation present palliative treatment methods (Wei & Harari 2012). There are also recent reports on the use of frequent denosumab for intractable hypercalcaemia (Karupiah 2014). Chemotherapy with dacarbazine and several other regimens are mostly ineffective (Wei & Harari 2012), although radiofrequency ablation of metastatic parathyroid carcinoma may control disease with a survival benefits of up to 15 months (Iguchi et al. 2008, Lourencop et al. 2012). Recent guidelines suggest that adjuvant radiotherapy should be reserved as a palliative option (Wilhelm et al. 2016).

Molecular targeted treatment

Data are scarce with regard to molecular targeted treatment in these rare neoplasms. Loss of APC expression suggests the possibility for oncological intervention in patients with parathyroid carcinoma as cyclooxygenase-2 (COX-2) inhibitors have been shown to exhibit chemo-preventive features in colorectal cancer (Giardiello et al. 1993, Eisinger et al. 2007). Recently, it has been shown that truncated APC can target β-catenin for destruction, but only in the absence of COX enzyme activity, providing a possible molecular explanation for the promising results of COX-2 inhibition in colorectal cancer (Eisinger et al. 2007). In vitro, the COX-2 inhibitor NS-398 suppressed parathyroid-related peptide (PTHrP) production in colorectal cancer (Saito et al. 2007). Anti-PTH immunotherapy has been described in some case reports, demonstrating biochemical control and clinical improvement as well as reduction of the size of metastases (Betea et al. 2004, Horie et al. 2010). Despite these promising results, such therapy is not easily available.

Type 1 membrane-bound alpha-Klotho (Klotho) defines tissue specificity for the phosphaturic hormone fibroblast growth factor-23 (FGF23) by acting as a permissive co-receptor (Urakawa et al. 2006). FGF23 binds to binary complexes of an FGF receptor (FGF-R) and Klotho to suppress parathyroid hormone (PTH) secretion (Krajinsnik et al. 2007). Klotho activity has also been implicated as fundamental for the stimulation of PTH secretion during hypocalcaemic conditions (Imura et al. 2007), although the underlying mechanism has been challenged (Martuseviciene et al. 2011). An in vitro study has demonstrated that blocking a parathyroid-FGF23 signalling pathway, involving calcineurin, led to abolition of FGF23-induced suppression of PTH secretion (Olausonetal. 2013). Thus, this pathway could be another future molecular target pathway for the treatment of parathyroid carcinoma.

The epidermal growth factor receptor (EGF-R, ERBB1, HER1) and its ligands have been reported to play an important role in bone biology and in mediating the anabolic actions of intermittent PTH. Amphiregulin (AREG), a ligand of EGF-R, has been identified to be a target gene for PTH in vitro and in vivo (Schneider et al. 2009) mediating the anabolic bone effect of PTH. However, it is still unclear as to whether these putative effects of AREG are an essential component of PTH’s anabolic actions on bone (Jay et al. 2015).

In summary, the therapeutic options for unresectable parathyroid carcinoma are limited and largely ineffective. Surgery is the only curative treatment for parathyroid carcinoma. MTTs are relatively under-explored clinically to date, although anti-PTH immunotherapy in some case series seems to show some encouraging results.
Nevertheless, parathyroid carcinoma may be relatively indolent in terms of progression, and aggressive medical control of the associated hypercalcaemia is an essential part of its management.

Conclusions

MTT either alone or in combination with standard treatments has shown promising results when current therapies such as surgery, chemotherapy, and/or radiotherapy have failed to treat malignant adrenal, pituitary and parathyroid tumours. For progressive metastatic PCs/PGLs, PRRT (including 131I MIBG) as well as sunitinib have shown preliminary evidence of anti-tumour activity as either first-line treatment or following progression with currently available treatments, and in combination with TKIs, mTOR inhibitors and other agents. Combination treatment of chemotherapy with TKIs or immunotherapy could be considered in patients with ACCs who have failed standard treatment, albeit with significant adverse events. Malignant and aggressive pituitary adenomas may respond to PRRT either as single agents or in combination with temozolomide. There are as yet limited data on the role of MTT in parathyroid carcinoma. Nevertheless, MTT appears to be the future for the management of these rare malignant endocrine tumours allowing for a more customised and individualised approach to the therapy. Combination strategies targeting more than one signalling pathway are most promising, at least in vitro. Immunotheapy has also yet to be introduced into these therapeutic paradigms. Multicentre trials are needed to obtain robust conclusions and the development of centralised databases accessible to different centres could help to better interrogate these tumours in larger populations.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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