Somatostatin receptor ligands and resistance to treatment in pituitary adenomas

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Abstract
Somatostatin (SST), an inhibitory polypeptide with two biologically active forms SST14 and SST28, inhibits GH, prolactin (PRL), TSH, and ACTH secretion in the anterior pituitary gland. SST also has an antiproliferative effect inducing cell cycle arrest and apoptosis. Such actions are mediated through five G-protein-coupled somatostatin receptors (SSTR): SSTR1–SSTR5. In GH-secreting adenomas, SSTR2 expression predominates, and somatostatin receptor ligands (SRLs; octreotide and lanreotide) directed to SSTR2 are presently the mainstays of medical therapy. However, about half of patients show incomplete biochemical remission, but the definition of resistance *per se* remains controversial. We summarize here the determinants of SRL resistance in acromegaly patients, including clinical, imaging features as well as molecular (mutations, SSTR variants, and polymorphisms), and histopathological (granulation pattern, and proteins and receptor expression) predictors. The role of SSTR5 may explain the partial responsiveness to SRLs in patients with adequate SSTR2 density in the cell membrane. In patients with ACTH-secreting pituitary adenomas, i.e. Cushing’s disease (CD), SSTR5 is the most abundant receptor expressed and tumors show low SSTR2 density due to hypercortisolism-induced SSTR2 down-regulation. Clinical studies with pasireotide, a multireceptor-targeted SRL with increased SSTR5 activity, lead to approval of pasireotide for treatment of patients with CD. Other SRL delivery modes (oral octreotide), multireceptor-targeted SRL (somatoprim) or chimeric compounds targeting dopamine D2 receptors and SSTR2 (dopastatin), are briefly discussed.

Introduction
The discovery of somatostatin or somatotropin release-inhibiting factor (SST) as an inhibitory polypeptide hormone was soon followed by its therapeutic use. Understanding SST action at each of the five subtypes of G-protein-coupled somatostatin receptors (SSTR), SSTR1–SSTR5, and the description of the downstream signaling and function highlighted both hormone secretion inhibitory activity and an antiproliferative role. Furthermore, identification of key SST molecular structural characteristics provided the rationale for its modification and design of novel analogs that function as specific ligands to cognate receptors.

Somatostatin receptor ligands (SRLs) are structurally similar to SST and have been used to treat growth hormone (GH)-secreting and gastroenteropancreatic neuroendocrine tumors. Subsequently, SRLs have been...
tested in other pituitary tumors such as thyrotropin (TSH) and adrenocorticotropic (ACTH)-secreting adenomas. The currently approved formulations of SRLs are octreotide (s.c. and long-acting repeatable/release (LAR)) and lanreotide (slow release (SR) or aqueous gel formulation autogel (ATG)) for acromegaly, and pasireotide for Cushing’s disease (CD). An oral octreotide formulation and a long-acting pasireotide LAR are under clinical evaluation.

Response to SRLs (control of hormonal hypersecretion and tumor shrinkage) is variable in both acromegaly and CD patients. This review summarizes SST structure, function, receptor pathways, and various SRLs (clinically available or being developed). Mechanisms (molecular, histopathological, and clinical) that are currently known to be associated with pituitary tumor resistance to SRL therapy are also discussed.

**SST structure, synthesis, and function**

SST is a cyclic peptide with a circulating half-life of <3 min and two biologically active forms (Brazeau et al. 1973): a 14 amino acid (AA) structure – SST14 – and a polypeptide with 14 AAs and a NH₂-terminal extension – SST28 (Fig. 1). SST is generated from larger precursor molecules called prepro-SST (116 AAs), which is then processed to pro-SST (92 AAs) and subsequently to SST14 and SST28. The name SST originates from a supposed ‘specific’ function as an inhibitor of somatotropin (GH) release, thus SST. SST can also directly stimulate GH secretion at low concentrations (Córdoba-Chacón et al. 2012a). SST production occurs not only in the CNS and peripheral nervous system, pancreas, and gut, but also in the placenta, kidneys, thyroid, retina, adrenals, and submandibular glands. In the CNS, SST acts as both a stimulatory and an inhibitory neurotransmitter. However, it has a key inhibitory action in the secretion of GH (Brazeau et al. 1973), prolactin (PRL; Vale et al. 1974), TSH (Siler et al. 1974), and ACTH (Richardson & Schonbrunn 1981) from the anterior pituitary gland. At the peripheral nervous system level, SST plays a regulatory role in the gastrointestinal tract inhibiting flow from the gallbladder, bowel motility and gastric emptying, smooth muscle contraction, and nutrient absorption from the intestine as well as in the exocrine pancreas. It also inhibits the release of glucagon (Boden et al. 1986), insulin (Alberti et al. 1973), and pancreatic polypeptide (Koerker et al. 1974) as well as cytokine release from immune cells (van Hagen et al. 1994).

Although the main effect of SST is to block exocytosis and hormone secretion, in vitro and in vivo studies have suggested suppression of GH and ACTH transcription through SSTR2 (Castillo et al. 2011, Ben-Shlomo et al. 2013).

**Structure of SSTR**

The biological actions of SST are mediated through five seven-domain G-protein-coupled receptors, SSTR1–SSTR5. The gene encoding SSTR2 contains an intron; the transcribed mRNA is spliced to encode two variants, a long (SSTR2A) form and a shorter (SSTR2B) form, which differ only in the length of their C-terminus tail (Patel et al. 1993). The human pituitary expresses only SSTR2A.
Truncated SSTR5 variants in mice, rats, and humans from normal and tumoral pituitary samples have also been characterized (Durán-Prado et al. 2009, Córdoba-Chacón et al. 2010). Native SST14 binds to SSTR1–SSTR4 with higher affinity, while SST28 is SSTR5 selective (Patel et al. 1994).

SSTR subtypes have 42–60% identical AA sequences (Reisine & Bell 1995). The extracellular loop 2, between domains 4 and 5, and the hydrophobic and charged AAs within transmembrane domains 3, 6, and 7 are essential for the interaction with SST and second messenger system activation (Kaupmann et al. 1995, Strnad & Hadcock 1995).

**SSTR expression in normal pituitary**

Fetal normal pituitary expresses all five SSTRs; however, the adult human pituitary gland expresses SSTR1, SSTR2, SSTR3, and SSTR5. SSTR4 is expressed in extremely low levels (Reubi et al. 2001, Vieira Neto et al. 2009, Ben-Shlomo & Melmed 2010). SSTR5 is the highly expressed SSTR subtype in normal human pituitaries followed by SSTR2, SSTR1, SSTR3, and SSTR4 (Vieira Neto et al. 2009). Normal human pituitaries express more SSTR1 and less SSTR2 when compared with somatotropinomas, whereas there are no significant differences in the expression of SSTR5, SSTR3, and SSTR4 (Vieira Neto et al. 2009). High-dose SST induces SSTR1, SSTR2, and SSTR5 expression in normal primate somatotropes (Córdoba-Chacón et al. 2012b). On the other hand, low-dose SST increases GH release and SSTR1 mRNA expression, but decreases SSTR5 expression (Córdoba-Chacón et al. 2012b). GH and TSH are inhibited by SSTR1, SSTR2, and SSTR5, while PRL secretion is predominantly inhibited by SSTR5 (Shimon et al. 1997a,b). Increased ACTH in Sstr2 knockout mice indicates a possible regulatory role for SSTR2. However, SSTR5 displays a more potent suppressive action on ACTH release (Table 1; Hofland et al. 2005).

**SSTR expression in pituitary adenomas**

The same SSTR subtypes as in normal pituitary are also found in pituitary adenomas; however, SSTRs show different expression level and activity depending on the type of adenoma cell (Table 1; Taboada et al. 2007, 2008, Ben Shlomo & Melmed 2010).

**SSTR expression in GH-secreting pituitary adenomas**

Acromegaly is predominantly caused by a GH-secreting pituitary adenoma, resulting in high circulating GH and insulin-like growth factor 1 (IGF1) concentrations (Melmed 2006). SSTR2 and SSTR5 are predominantly expressed, both at the mRNA level (Jaquet

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**Table 1** Biochemical features of human somatostatin receptors. Data derived from Reubi et al. (2001), Hofland & Lamberts (2003), Florio (2008), Lania et al. (2008) and Ben-Shlomo et al. (2009)

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*aThe values represent the percentage of tumors expressing the SSTR subtype among the tumors screened including studies using solution hybridization, ribonuclease protection assay, RT-PCR, quantitative RT-PCR, northern blotting, in situ hybridization, and immunohistochemistry. NFA, clinically non-functioning pituitary adenoma.
et al. 2000) and at the protein level (Reubi et al. 2001). SSTR2 is expressed in ~95% of GH-secreting adenomas, while SSTR5 is found in ~85%. SSTR1 and SSTR3 are found in much fewer cases (40%), while SSTR4 is almost never identified (Table 1; Reubi et al. 1994, Nielsen et al. 2001). Moreover, SSTR5 was identified as the most abundant subtype, followed by SSTR2, SSTR3, SSTR1, and SSTR4 using quantitative evaluation of the SSTR subtypes (Taboada et al. 2007). Co-secreting GH and PRL adenomas have increased SSTR5 expression (Shimon et al. 1997a, b, Hofland et al. 2004).

**SSTR expression in ACTH-secreting pituitary adenomas** CD is caused by a pituitary corticotrope adenoma responsible for ACTH hypersecretion with subsequent adrenal overproduction of cortisol. The majority of corticotrope adenomas (>85%) express SSTR2 and SSTR5 mRNAs, and to a lesser extent SSTR1 mRNA (63%) (Miller et al. 1995). The membrane density of SSTR subtypes, particularly SSTR2, is affected by hypercortisolism (de Bruin et al. 2009). By contrast, SSTR5 expression appears to be relatively unaffected by high cortisol levels. Therefore, in patients with active CD, SSTR5 is predominantly expressed over SSTR2 (de Bruin et al. 2009). After eucortisolemia restoration, SSTR2 expression recovers, becoming similarly abundant as SSTR5, thus improving treatment responsiveness (Fig. 2; van der Pas et al. 2013). Human SSTR2 and SSTR5 overexpression in mouse corticotrope adenoma AtT20 cells demonstrates SST regulation of ACTH secretion and the ligand-free effects and constitutive activity of such receptors (Ben-Shlomo et al. 2009).

**SSTR expression in other pituitary adenomas** PRL-secreting pituitary adenomas mainly express SSTR1 and SSTR5, whereas SSTR2 is expressed at a lower level. SSTR5 has a high PRL-suppressive effect (Table 1; Jaquet et al. 1999).

TSH-secreting adenomas are rare tumors, already macroadenomas at diagnosis with mass effect symptoms and signs of hyperthyroidism. SSTR2 seems to be the predominant receptor, but co-expression with SSTR5 has corticotrope adenomas is reversible after achieving eucortisolemia; use of SSTR2 receptor ligands may induce improved biochemical responsiveness (van der Pas et al. 2013).

Figure 2
Hypercortisolemia in active Cushing’s disease (CD) hampers ACTH suppression by somatostatin or SSTR2 receptor ligand treatment due to down-regulation of SSTR2 expression in corticotrope pituitary adenomas (de Bruin et al. 2009). Cortisol-mediated SSTR2 down-regulation in
also been reported (Table 1; Yoshihara et al. 2007, Gatto et al. 2012).

Non-functioning pituitary adenomas (NFAs) show a variable SSTR subtype expression profile, whereby SSTR2 and SSTR3 seem to be the predominant subtypes (Table 1; Zatelli et al. 2004, Vieira Neto et al. 2013).

SSTR signaling pathway

A conformational change of the receptor after SST binding leads to activation of an associated heterotrimeric G-protein complex (consisting of α-, β-, and γ-subunits) and exchange of GTP for GDP on the α-subunit. Through pertussis toxin-sensitive inhibitory Gi protein (G<i>i</i>) activation, adenylyl cyclase activity is hampered in all five SSTRs. This results in reduction of intracellular cAMP and calcium (Ca<sup>2+</sup>) levels, with hyperpolarization of potassium (K<sup>+</sup>) channels as well as inhibition of Ca<sup>2+</sup> influx closing voltage-sensitive Ca<sup>2+</sup> channels (Patel et al. 1994). The lack of intracellular Ca<sup>2+</sup> impedes intracellular vesicle movement, exocytosis, and hormone secretion (Fig. 3). Depending on the specific SSTR subtype, the final pathway and effect on cellular function may be different. In addition, SSTR signaling is further modulated by receptor endocytosis and trafficking (Fig. 4; Hofland & Lamberts 2003, Lesche et al. 2009).

Mechanism of action for pituitary tumor growth inhibition

SST has a potent antiproliferative effect through both direct and indirect mechanisms.

Figure 3

Schematic of the intracellular signaling pathways modulated by SSTR2 after somatostatin (SST) or SRLs action. From left to right, the anti-proliferative pathway is activated through three different phosphotyrosine phosphatases (PTPs), SHP1, SHP2, and PTP1 (Pan et al. 1992). SHP2 activates Src that directly interacts with PTP1 inducing its phosphorylation and activation (Lopez et al. 1997). Then, Ras and Rap1-GTP inhibits the phosphatidylinositol-3-kinase (PI3K) target Akt. This, in turn, activates the MAPK pathway stimulating the ERK1/2, and also p38, which have as targets Elk1, and the activating transcription factor 2 (ATF2) (Sellers et al. 2000). In addition, the glycogen synthase kinase 3 (GSK3)<i>b</i>) is phosphorylated and inhibited. The results are upregulation of p21<sup>cip1</sup> and p27<sup>Kip1</sup>, which will arrest cell cycle at the G1/S transition phase (Ben-Shlomo & Melmed 2010, Theodoropoulou et al. 2010). Activated SHP1 also triggers intracellular pro-apoptotic signals involving the induction of caspases activation and p53/Bax. GSK3<i>b</i>) activates the tumor suppressor tuberin TSC2/TSC1 complex, which in turn inhibits the mammalian target of rapamycin (mTOR) also inducing cell apoptosis. Additionally, SHP1 associates with nitric oxide synthase (NOS) and dephosphorylates it, leading to NOS activation and nitric oxide (NO) production. NO activates soluble guanylate cyclase (GC), which converts GTP to cGMP. Increased cGMP inhibits cell growth (Ben-Shlomo & Melmed 2010). SST also induces ZAC1 expression through a mechanism involving Gai, SHP1, GSK3<i>b</i>), and the Zac1 activator p53. Zac1 is capable of inducing apoptosis and cell cycle arrest (Theodoropoulou et al. 2010). Finally, SST has key anti-secretory effects increasing K<sup>+</sup> efflux and membrane hyperpolarization, which in turn closes voltage-dependent Ca<sup>2+</sup> channels decreasing intracellular Ca<sup>2+</sup> influx and concentration. Adenylyl cyclase inhibition also lowers cAMP levels and PKA activity hampering hormone secretion (Patel et al. 1994). Activated pathway, black arrows; inhibited pathway, red arrows.
Direct antiproliferative mechanisms The G\textsubscript{ai} subunit is activated (after SST binding at SSTR2), inducing the antiproliferative second messenger pathway associated with protein tyrosine phosphatases (PTPs; Pan et al. 1992). This pathway involves activation of the cytosolic Src homology 2 (SH2) domain, SHP1 (PTPN6), and SHP2 (PTPN11), and the membrane-anchored PTP\textsubscript{h} (DEP1 (PTPRJ)) (Lopez et al. 1997; Fig. 3). This, in turn, activates the serine/threonine MAPK pathway. The MAPK pathway usually mediates the mitogenic action of growth factors, cytokines, and hormones. However, depending on the cell system, it can also halt cell growth in order to promote differentiation. Thus, SSTR-induced MAPK activation causes cell cycle arrest with up-regulation of two key cell inhibitors: p21\textsuperscript{Cip1} and p27\textsuperscript{Kip1}, which will prevent the formation of the cyclin-dependent kinase (CDK) complexes, arresting the cell cycle at the G1/S transition phase (Fig. 3; Ben-Shlomo & Melmed 2010, Theodoropoulou et al. 2010). Activated SHP1 and the glycogen synthase kinase 3\textbeta (GSK3\textbeta) also trigger intracellular pro-apoptotic signals (Fig. 3). Additionally, SHP1 associates with nitric oxide synthase (NOS) and dephosphorylates it to increase cGMP levels and inhibit cell growth (Fig. 3; Ben-Shlomo & Melmed 2010).

Although CDK inhibitor 1B (p27\textsuperscript{Kip1}) is an important target of the antiproliferative action of SST, the rat pituitary tumor GH3 cells do not express p27 Kip1 (Cdkn1b), but shows decreased cell proliferation through SSTR2 activation. In these cells, SSTR2 induces ZAC1 (ZACN) expression through a mechanism involving G\textsubscript{ai}, SHP1, GSK3\textbeta, and the ZAC1 activator p53 (Fig. 3; Theodoropoulou et al. 2010). ZAC1 is a zinc finger protein, predominant in normal adenohypophysis, capable of inducing apoptosis and cell cycle arrest (Theodoropoulou et al. 2010). Loss of expression frequently occurs in NFAs (Theodoropoulou et al. 2010, Vieria Neto et al. 2013). ZAC1 target genes include the pituitary adenylate cyclase-activating polypeptide type 1 receptor (PAC1R), peroxisome proliferator-activated receptor \gamma (PPAR\textgamma), and p21\textsuperscript{Cip1} and p57\textsuperscript{Kip2} via coactivation of p53 and p73 (Theodoropoulou et al. 2010). ZAC1 gene expression increases if phosphoinositide 3-kinase (PI3K) is inhibited using octreotide treatment, leading to inhibition of 3-phosphoinositide-dependent protein kinase 1 (PDK1).
and the protein Ser/Thr kinase (Akt) activities that, in turn, result in GSK3β activation and increased p53 transcriptional activity that up-regulates ZAC1 expression (Fig. 3; Theodoropoulou et al. 2010).

By activation of SHP1-mediated E-cadherin dephosphorylation, SSTR2 activity also suppresses tumor invasiveness restoring E-cadherin function, which is reduced with cell dedifferentiation (Benali et al. 2000).

Antiproliferative effects of SSTR5 are mediated through permeability transition pore (PTP)-independent pathways (Fig. 5). SSTR5 uses its inhibitory action on phospholipase C (PLC) and inositol 1,4,5-trisphosphate (IP3) to inhibit the intracellular release of Ca\(^{2+}\) as well as the extracellular Ca\(^{2+}\) influx (Fig. 5; Wilkinson et al. 1997). In addition, SSTR5 blocks cell proliferation inhibiting MAPK and c-fos (Fig. 5; Cordelier et al. 1997). Cells expressing both SSTR2 and SSTR5 have more efficacious adenylate cyclase inhibition, ERK1/2 activation, and p27\(^{kip1}\) induction after SSTR2 activation by a selective agonist, when compared with cells expressing SSTR2 alone. This finding suggests that amplification of the cell proliferation pathway inhibition may be actually achieved through receptor heterodimerization (Rocheville et al. 2000). Interestingly, SSTR5 truncated variants showed intracellular distribution and preserved functionality with ligand-induced Ca\(^{2+}\) and cAMP despite being truncated, suggesting distinct roles after SST action (Córdoba-Chacón et al. 2010).

**Indirect antiproliferative mechanisms** IGF1 reduction after inhibition of GH secretion is the most important indirect mechanism of cell growth inhibition by SST (Shimon et al. 1997b). Activation of hepatic SSTR2 and SSTR3 down-regulates IGF1 transcription by dephosphorylation and inactivation of GH-induced STAT5b (Muray et al. 2004). Additional suppression of the secretion or synthesis of epidermal growth factor, platelet-derived growth factor, and transforming growth factor α by SST contributes to a decrease in tumor growth (Florio 2008).

Further indirect antiproliferative effects are endothelial cell proliferation reduction with inhibition of angiogenesis. Three signaling pathways are identified as responsible for the antiangiogenic activity of SST:

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**Figure 5**
Schematic of the intracellular signaling pathways modulated by SSTR5 after somatostatin (SST) or SRLs action. In contrast to SSTR2, the antiproliferative mechanism is activated through PTP-independent pathways. After inhibition of adenylate cyclase by Gαi subunit activation, SSTR5 affects phospholipase C (PLC) which in turn cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). The latter is released into the cytosol, where it binds to its receptor (IP3R) at endoplasmic reticulum and release intracellular Ca\(^{2+}\) whereas DAG recruits and activates PKC, which in turn open voltage-gated Ca\(^{2+}\) channels. SSTR5 uses its inhibitory action on PLC and IP3 to inhibit intracellular release of Ca\(^{2+}\) as well as extracellular Ca\(^{2+}\) influx (Wilkinson et al. 1997). In addition, SSTR5 blocks cell proliferation inhibiting MAPK and c-fos (Cordelier et al. 1997). Activated pathway, black arrows; inhibited pathway, red arrows.
i) Down-regulation of the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) transcription. VEGF drives the development of new vessels in the growing tumor as well as inhibits endothelial nitric oxide and the following attenuation of nitric oxide, a second messenger that plays a pivotal role in angiogenesis (Florio et al. 2003). SST also decreases VEGF cell secretion and viability (Zatelli et al. 2007). ii) Inhibition of endothelial cell activity (proliferation, migration, and invasion). The antiproliferative effect is mediated by inhibition of ERK1/2 activity and endothelial NOS (eNOS). The anti-invasive effect of SST is dependent on the inhibition of MAPK, the small G-protein RAC (ATK1), and the expression of metalloproteases such as MMP2 (Florio et al. 2003). iii) Inhibition of monocyte activation. SST inhibits monocyte migration and their recruitment in the areas where new vessels are forming (Florio 2008).

### Somatostatin receptor ligands

The potent antisecretory properties of SST have made it an important pharmacological target for treatment of hormonal hypersecretion. However, its short half-life, the multiple and simultaneous actions in different organs, the need of parenteral administration, and the post-infusion rebound in GH, insulin, and glucagon release hampered its clinical use. SRLs were developed after manipulation of key SST structural characteristics, which allowed for several improvements over the above disadvantages.

#### Single-receptor-targeted SRLs

**Octreotide and lanreotide** Essential structural features of SST include the β-turn comprising 7–10 AAs, as well as the cysteine–cysteine–cysteine (Cys–Cys) bridge between position 3 and terminal position 14, with the most critical cleavage at tryptophan on position 8, which leads to completely inactive fragments (Fig. 1; Brazeau et al. 1973). The clinically available synthetic SRLs, SMS 201-995 or octreotide (Bauer et al. 1982) and BIM-23014 or lanreotide (Taylor et al. 1988), were developed by capturing the Cys–Cys bridge and stabilizing the β-turn incorporating a D-Trp (Fig. 1). Both peptides display selective high-affinity binding to SSTR2, with a moderate affinity for SSTR3 and SSTR5, and no binding to SSTR1 and SSTR4 (Table 2; Ben-Shlomo & Melmed 2008). They have enhanced metabolic stability (half-life of 2 and \(1\) h respectively), a small volume of distribution, and low clearance, all resulting in a longer duration of exposure and consequently a long-lasting biological activity compared with SST. Furthermore, rebound hypersecretion does not occur, making these ligands optimal for clinical use (Ben-Shlomo & Melmed 2008).

LAR formulation results from mixing octreotide with microspheres of biodegradable glucose polymers and diluent (carboxymethylcellulose sodium, mannitol, and water). Therapeutic concentrations of the drug in this suspension can be maintained for 24–42 days (Ben-Shlomo & Melmed 2008). Octreotide LAR is 41–65% protein bound and 11–32% of the administered

#### Table 2 Somatostatin receptor ligands and chimeric compounds

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<th>Dose</th>
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<td>For experimental research</td>
<td>–</td>
<td>Piöckinger et al. (2012)</td>
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<td>Chimeric</td>
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<td>Dopastatin</td>
<td>2 and DA2R</td>
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DA2R, dopamine D2 receptor; SR, slow release; LAR, long-acting release.
drug is eliminated unchanged in the urine. Within 1 h of a single i.m. dose of octreotide LAR of 10–30 mg, an initial peak in serum concentrations occurs presumably from drug adsorbed to the carrier microspheres; then, concentrations decline within 12 h of drug administration, remaining subtherapeutic until day 7, before increasing in a dose-dependent manner to a plateau in about 14 days (Table 2). The plateau remains stable until days 35–60 and then steadily declines. Octreotide LAR (20 or 30 mg) at 4-week intervals reaches the steady state after three injections (BBen-Shlomo & Melmed 2008). Therapeutic drug concentration ranges between 1 and 3 μg/l.

Lanreotide is also available in two long-acting formulations. Sustained-release lanreotide (lanreotide SR), prepared in microspheres of biodegradable lactide/glycolide copolymers, is administered by i.m. injection at a dose of 10–30 mg every 7–14 days, and 60 mg every 21–28 days. A second formulation is lanreotide autogel, consisting of a solution that is a long-acting viscous aqueous formulation of lanreotide, which is supplied in ready-to-use prefilled syringes (does not require reconstitution) intended for deep s.c. injection. It has a linear pharmacokinetic profile and is administered every 28–56 days (Table 2; Ben-Shlomo & Melmed 2008).

**Oral delivery** An oral formulation for octreotide (Octreolin; Chiasma) became feasible with the transient permeability enhancer technology that enables the drug to be absorbed by the intestine. A phase II study demonstrated equivalent pharmacokinetic parameters using 20 mg oral octreotide or 100 μg s.c. octreotide, supporting oral octreotide as an alternative to parenteral formulation to treat acromegaly patients (Table 2; Tuvia et al. 2012). A phase III trial is ongoing to investigate the efficacy and safety of octreolin in patients with acromegaly (http://clinicaltrials.gov/ct2/show/NCT01412424).

**Multireceptor-targeted SRLs and chimeric molecules**

**Pasireotide** Essential functional groups of the SST peptide with high binding affinity to SSTRs were detected using the alanine scanning technology. Incorporation of four synthetic and two essential AAs of SST in the form of a novel basic trans-((L)-hydroxyproline aminoethyl-urethane extension, phenylglycine, O-benzyl-tyrosine, and d-Trp to corresponding positions into a stable cyclohexapeptide template resulted in SOM230 (pasireotide), a multiligand SRL (Fig. 1; Bruns et al. 2002, Fleseriu & Petersenn 2013). SOM230 displays a 40, 30, and 5 times higher binding affinity to SSTR5, SSTR1, and SSTR3 respectively, and a 2.5 times lower binding affinity to SSTR2, compared with octreotide (Bruns et al. 2002). Due to longer half-life (~24 h), the once- or twice-daily dosing regimen maintains steady-state concentrations (Table 2; Boscaro et al. 2009, 2013, Fleseriu & Petersenn 2013).

Pasireotide modulates SSTR trafficking in a different manner than octreotide, resulting in rapid recycling of SSTR2 to the plasma membrane after endocytosis (Lesche et al. 2009). While octreotide and SST14 phosphorylated at least six carboxy-terminal serine and threonine residues of SSTR2, pasireotide stimulates selective phosphorylation of S341 and S343 residues, which was followed by its internalization (Nagel et al. 2011). This accelerated recycling may counteract the desensitization of SSTR2 (Fig. 4).

A LAR form of pasireotide was developed using biodegradable polymers similar to octreotide LAR (Table 2). Pasireotide LAR is undergoing clinical trials (http://clinicaltrials.gov) for CD (NCT01374906), acromegaly (NCT01137682, NCT01673646, NCT00600886, and NCT00446082), and NFAs (NCT01283542).

**Somatoprim** DG3173 (somatoprim (Fig. 1)) is a novel SRL that binds to SSTR2, SSTR4, and SSTR5 (Table 2). *In vitro* studies reported a higher response to somatoprim than octreotide, including an additional 38% of pituitary adenomas resistant to octreotide. Furthermore, the lower insulin-suppressing activity may be associated with less hyperglycemic effect than the other SRLs (Piöckinger et al. 2012).

**Dopastatin** The combination of individual SSTR2 and dopamine D2 receptor (DAR2) agonists shows an additive effect on suppressing GH secretion in both rat and human GH-secreting pituitary cells (Ren et al. 2003a). BIM-23A387 or dopastatin is a chimeric compound containing structural elements of both SST and dopamine in a single molecule, retaining potent and selective binding to both SSTR2 and DAR2 (Table 2; Ren et al. 2003a). However, clinical studies with dopastatin have been halted due to insufficient SST-like activity.

**SRL effects in pituitary tumors**

**GH-secreting pituitary tumors**

**Octreotide and lanreotide** SRLs represent the mainstay of medical therapy for acromegaly (Melmed 2006, Melmed et al. 2009). In contrast to the tachyphylaxis of physiological GH secretion after continuous receptor
activation seen within hours to days (Hofland & Lamberts 2003), therapeutic escape has not been observed in acromegaly, even after prolonged treatment. Several clinical studies observed normalization of GH and IGF1 levels and amelioration of clinical symptoms in about 60–70% of acromegaly patients (Ben-Shlomo & Melmed 2008, Melmed et al. 2009, Fleseriu 2013). However, prospective studies that included patients, not preselected for SRL efficacy, have shown much lower rates of biochemical control (Mercado et al. 2007, Melmed et al. 2010, Bronstein et al. 2012). The efficacies of octreotide LAR and lanreotide autogel seem to be similar (Ben-Shlomo & Melmed 2008, Murray & Melmed 2008, Melmed 2009). Dose escalation provide additional biochemical control in patients who were inadequately controlled with conventional starting SRL doses, without significantly changing safety and adverse events (Fleseriu 2011).

Tumor shrinkage occurs in about 33% (10–77%) of patients treated with lanreotide autogel (Mazzotti & Giustina 2010) and about 53% (45–61%) and 66% (57–74%) of patients with octreotide and octreotide LAR respectively (Giustina et al. 2012). As expected, naïve patients experience a better response compared with those previously treated with radiotherapy and surgery (Mazzotti & Giustina 2010).

Overall, the true efficacy of SRLs and all medical therapies in general are somewhat difficult to assess because of varying study entry criteria (de novo, postsurgery, or preselected sensitivity to SRLs) and changing biochemical cutoff goals over time (Bronstein et al. 2012, Fleseriu 2013). However, only a small minority (<10%) should be considered to be fully resistant to SRLs (Colao et al. 2011).

Advances in combination medical therapy offer new perspectives for those patients who are partially resistant to available SRLs.

**Pasireotide** Pasireotide induces a dose-dependent decrease in GH and IGF1 levels in rats, dogs, and monkeys (Bruns et al. 2002). In human mixed GH/PRL-secreting adenomas, pasireotide suppressed both PRL and GH, which was also confirmed in vitro using Hmgα2 overexpressing mice (Hofland et al. 2004, Fedele et al. 2007). In a phase II study in acromegaly patients, after 4 weeks of 200–600 μg s.c. pasireotide twice daily, 19% of patients achieved biochemical control (GH ≤ 2.5 μg/l and normal IGF1). This increased to 27% after 3 months and 39% of patients had a significant (>20%) reduction in pituitary tumor volume (Petersenn et al. 2010). In a large phase III clinical trial, pasireotide LAR achieved disease control at 12 months in 31 vs 19% of patients treated with octreotide LAR. Notably, this study included a large majority of patients with no previous history of pituitary surgery (Bronstein et al. 2012). Furthermore, switching patients who were not controlled on octreotide LAR to pasireotide LAR enabled 21% of patients to achieve biochemical control (Fleseriu et al. 2012). However, the frequency and degree of hyperglycemia and diabetes in all pasireotide studies were significantly higher compared with octreotide.

**SRLs in ACTH-secreting pituitary adenomas**

**Octreotide and lanreotide** Octreotide is able to suppress ACTH levels in corticotropinoma primary cell culture in vitro, but is without effect in vivo. Octreotide and lanreotide are SSTR2-preferring ligands: their low efficacy in reducing ACTH levels is most probably due to low SSTR2 expression of these tumors in hypercortisolemic states (Fig. 2; de Bruin et al. 2009).

**Pasireotide** ACTH secretion in human corticotropinoma primary cell cultures as well as in mouse AtT20 corticotropinoma cells resistant to octreotide (due to glucocorticoid-induced SSTR2 down-regulation) is suppressed by pasireotide. In stable AtT20 cells overexpressing SSTR2 and SSTR5, short- and long-term enhanced pasireotide action and its potency were not affected by SSTR2 abundance, SSTR2 antagonist, or octreotide co-treatment (Ben-Shlomo et al. 2009). Studies in dogs with CD showed both ACTH and cortisol reduction with symptoms ameliorated after 6 months of pasireotide treatment (Castillo et al. 2011). Of the 29 patients in the primary efficacy analysis of the phase II study in CD, 22 (76%) showed a reduction in urine free cortisol (UFC) levels, after 15 days of treatment with 600 μg pasireotide twice daily. Of them, 17% achieved normal UFC levels; serum cortisol levels and plasma ACTH levels were also reduced. Steady-state plasma concentrations of pasireotide were achieved within 5 days of treatment; interestingly, responders appeared to have higher exposure than non-responders (Boscaro et al. 2009). A large phase III study showed normalization of UFC in 15 and 26% for the 600 and 900 μg doses respectively, including normalization of UFC in ~50% of the mild cases (defined as UFC less than twofold of the upper limit of normal (ULN) at baseline) on 900 μg twice a day treatment. However, pasireotide was associated with hyperglycemia-related adverse events in 118 out of 162 cases (Colao et al. 2012). The therapeutic effect of pasireotide is through activation of SSTR5 and the drug also enhances ACTH and cortisol suppression via
restored SSTR2 expression (Fig. 2; van der Pas et al. 2013). In addition to clinical improvement and UFC reduction, salivary cortisol can be a useful parameter to monitor treatment responsiveness (Biller et al. 2013). Pasireotide is the first pituitary-targeted therapy available for treatment of CD (Colao et al. 2012, Fleseriu & Petersenn 2012, Fleseriu & Petersenn 2013, Tritos & Biller 2013). Furthermore, interaction between SRLs and dopamine receptor agonists may allow for synergistic suppression of ACTH, but the role of combination therapy must be further characterized (Fleseriu 2012, Fleseriu & Petersenn 2013).

**SRLs in other pituitary tumors**

Although long-term data are limited, most patients with TSH-secreting pituitary adenomas respond well to either octreotide or lanreotide after surgery. Ooctreotide induces tumor shrinkage in 30–50% of TSH-secreting pituitary adenomas (Ben-Shlomo & Melmed 2010).

Prolactinomas with predominant SSTR5 expression respond weakly to the SSTR2-directed ligands octreotide or lanreotide; however, in vitro experiments show a high PRL-suppressive effect using a selective SSTR5 agonist (Shimon et al. 1997a,b, Jaquet et al. 1999).

NFAs predominantly express SSTR2 (Table 1); however, the efficacy of SRLs remains to be proven. Tumor shrinkage has been sporadically reported in 12% of patients under SRL treatment. The mechanism may be related to the suppressive action of VEGF and PI3K inhibition.

**Definition of resistance to SRLs**

Definition of SRL resistance in pituitary tumor therapy should take treatment characteristics, biochemical control, and tumor shrinkage response into consideration (Colao et al. 2011).

**GH-secreting pituitary adenomas**

Criteria for biochemical remission in acromegaly include normal IGF1, random GH <1 μg/l, and a nadir GH after 75-g oral glucose tolerance test <0.4 μg/l (Giustina et al. 2010). Use of optimal GH and IGF1 assays and correction for gender, age, and BMI are essential. SRLs are rarely completely ineffective in GH-secreting pituitary tumors (Hofland & Lamberts 2003, Colao et al. 2011); however, there is no exact definition of partial resistance. In those patients without biochemical remission after 6 months of first-line treatment with SRLs, surgical debulking and then a second course of SRL may improve biochemical control (Colao et al. 2011).

‘Tumor resistance’ to SRLs is usually defined as <20% shrinkage from baseline volume (Colao et al. 2011) and could vary considerably between patients treated with first- or second-line therapy (Mazziotti & Giustina 2010, Giustina et al. 2012).

However, before considering SRL resistance, both duration (at least 12 months) and doses of SRL should be optimized (Fleseriu 2011). In recent studies, high-frequency (30 mg every 21 days) or high-dose (40 or 60 mg every 28 days) octreotide LAR achieved additional clinical, biochemical, and/or tumor shrinkage in 25% of patients with acromegaly (Giustina et al. 2009, Fleseriu 2011).

An algorithm proposed in March 2013 by the Acromegaly Consensus Group (Expert consensus document: a consensus on the medical treatment of acromegaly) for the medical management of acromegaly after surgery or as primary treatment strategy when surgery is inappropriate was recently published (Giustina et al. 2014). Therapeutic approaches for patients with SRL resistance were also reviewed in the guidelines and elsewhere (Fleseriu 2013, Gadelha et al. 2013, Giustina et al. 2014). For patients who do not have any response to SRL therapy, switching to pegvisomant treatment is optimal. For patients with inadequate response, administration of high-dose SRLs alone or in combination with the GH receptor antagonist pegvisomant and/or the dopamine agonist cabergoline should be considered (Colao et al. 2011, Fleseriu 2013, Gadelha et al. 2013, Giustina et al. 2014). Repeat pituitary surgery for tumor debulking and/or radiotherapy could also be entertained (Colao et al. 2011, Giustina et al. 2014).

**ACTH-secreting pituitary adenomas**

The definition of remission in CD is controversial, as there is no consensus on the most accurate assessment method or exact timeline, either postoperative or during medical treatment. Persistent immediate postoperative morning serum cortisol levels of <2 μg/dl (~50 nmol/l) are associated with remission (Biller et al. 2008, Hameed et al. 2013), but delayed remission has also been noted. Normalization of hypercortisolemia (normal UFC and salivary cortisol) remains the main goal of therapy in CD (Biller et al. 2008, 2013).

**Determinants of SRL responsiveness**

**GH-secreting pituitary adenomas**

**Clinical predictors** Age at diagnosis has been identified as a possible clinical marker of tumor size and
aggressiveness (Table 3). Younger patients tend to have larger and more aggressive tumors than older patients with less SRL response. Female patients and lower circulating IGF1 and GH levels at diagnosis have also been associated with better SRL response (Brzana et al. 2013, Gadelha et al. 2013).

**Tumor characteristics on magnetic resonance imaging**

**Tumor size and invasiveness** Radiological evaluation usually reveals a lower percentage of microadenomas (<10 mm in size, 20%) than macroadenomas (≥10 mm, 80%) (Melmed 2006). The surgical cure rate depends on the tumor size at presentation and is higher for microadenomas (81%) than non-invasive (71%) or invasive (37%) macroadenomas. Tumor invasiveness was shown to hamper surgical resection and reduce cure probability both after surgery and in response to SRL (Melmed 2006, 2009). As expected, patients treated with first-line therapy with SRLs achieved better shrinkage than those treated with second-line therapy after unsuccessful surgery (Mazziotti & Giustina 2010, Giustina et al. 2012). Tumor growth during SRL treatment is extremely rare (<2%).

**Adenoma intensity by magnetic resonance imaging** Adenoma hypointensity on T2-weighted MR images is associated with densely granulated, less invasive adenomas, with better SRL response (Heck et al. 2012). By contrast, T2 hyperintensity imaging suggests a sparse granulation pattern as assessed by immunohistochemistry, with more invasive and less SRL-responsive tumors (Heck et al. 2012). Therefore, T2 MR imaging signal may be able to identify responsiveness to SRL and prognosis

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MRI, magnetic resonance imaging.

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Table 3 Summary of the SRL resistance determinants

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in patients with active acromegaly after unsuccessful surgery (Table 3).

**Histopathological predictors**

Granulation pattern

Densely granulated GH-secreting pituitary adenomas are associated with a better SRL response than those with sparsely granulated adenomas (Brzana et al. 2013, Gadelha et al. 2013). The increased responsiveness of densely granulated tumors to SRLs has been related to high SSTR2A expression and high adenylate cyclase levels associated with the GSP mutation. Factors associated with lower SRL responsiveness in sparsely granulated adenomas are lower SSTR2A immunoreactivity (Brzana et al. 2013), somatic mutation in GH receptor interfering with post-translational processing, maturation, ligand binding, and signaling (Asa et al. 2007) as well as decreased E-cadherin immunoreactivity (Fougnier et al. 2010).

**SSTR2 expression**

SSTR2 expression is positively correlated with SRL response at both mRNA and protein levels (Table 3; Gadelha et al. 2013). Monoclonal anti-SSTR2 antibody immunostaining of paraffin-embedded tissues predicted SRL response in patients with acromegaly (Brzana et al. 2013). A high SSTR2 to SSTR5 ratio correlates with biochemical control under octreotide treatment (Gadelha et al. 2013).

**Ki-67 proliferation index**

Tumors with a high proliferation of marker Ki-67 are more invasive and SRL resistant (Table 3). Moreover, a lower Ki-67 index is identified in SRL-responsive treated patients, which could be due to their antiproliferative effects (Florio 2008).

**ZAC1**

ZAC1 is a tumor suppressor gene that was cloned from immortalized GH-secreting adenoma cells and mediates, at least in part, the antiproliferative action of octreotide (Theodoropoulou et al. 2010). A retrospective study of patients with acromegaly treated preoperatively with SRLs revealed a positive correlation between treatment response (both IGF1 normalization and tumor volume reduction) and ZAC1 immunoreactivity (Theodoropoulou et al. 2010).

**β-arrestins**

β-arrestins (1 and 2) are scaffold proteins recruited on the cell membrane to regulate uncoupling of G-protein-coupled receptors and are thought to participate in the desensitization process. In particular, β-arrestin 1 plays an important role in SSTR2 desensitization upon agonist activation (Fig. 4). Therefore, lower expression of β-arrestin 1 mRNA in GH- and PRL-secreting pituitary adenomas, compared with NFA, correlates with a reduced recycling rate of SSTR2, a higher amount of biologically active receptor exposed on the cell membrane, and better SRL response in terms of GH suppression, both in vitro and in vivo (Table 3; Gatto et al. 2013). Interestingly, pasireotide treatment resulted in lower β-arrestin recruitment with reduced SSTR internalization and trafficking in vitro (Lesche et al. 2009).

**Raf kinase inhibitory protein**

Raf kinase inhibitory protein (RKIP) functions as MAPK inhibitor in the SSTR2 signaling cascade. RKIP (PEBP1) expression is not correlated with SSTR2 expression; however, low levels of RKIP in GH-secreting pituitary adenomas correlated with poor octreotide treatment response (Fougnier et al. 2008).

**Molecular predictors**

**Mutations in SSTR genes and truncated SSTR5 variants**

The genes encoding SSTR1–SSTR5 are given in Table 1. Mutations in SSTR2 and SSTR5 genes are rarely encountered (Ballare et al. 2001), and their polymorphisms may influence basal and IGF1 levels (Table 3). However, polymorphic variants in SSTR2 and SSTR5 genes seem to have a minor role in determining SRL resistance in patients with acromegaly (Lania et al. 2008). The SSTR5 truncated variants may account for differential effects observed in different tissues upon SRL treatment (Table 3; Durán-Prado et al. 2009, Córdoba-Chacón et al. 2010).

**Mutations in aryl hydrocarbon receptor interacting protein gene**

Aryl hydrocarbon receptor interacting protein (AIP) is a tumor-suppressor gene, which has been shown to induce tumor shrinkage via ZAC1 (Gadelha et al. 2013). Patients with AIP gene germ line mutations are usually male, with young-onset, sparsely granulated GH-secreting pituitary tumors or GH/PRL mixed adenomas (Gadelha et al. 2013). Familial and sporadic mutations of the AIP gene predict an unfavorable SRL response. AIP expression, even without an AIP gene mutation, has also been associated with octreotide LAR resistance (Table 3). The mechanism seems to be independent of SSTR2 expression. However, SRL responsiveness when both markers are used together is predicted with an accuracy higher than that when each is used individually (Gadelha et al. 2013). In addition to biochemical control, tumor shrinkage after SRL treatment was significantly higher in the control group (40%) than in the AIP-mutated patients (0%) (Gadelha et al. 2013). Furthermore, SSTR5
staining is slightly higher in AIP-mutated tumors than in sporadic ones.

**SSTR desensitization** Many G-protein-coupled receptors, including SSTR1–SSTR5, show the ability to regulate their responsiveness to continued agonist exposure with different degrees of receptor internalization and degradation (Table 1; Hofland & Lamberts 2003). Although the acute administration of SST produces multiple inhibitory effects, the initial response diminishes with continued exposure to the peptide. However, in patients with acromegaly who respond well to SRLs, GH secretion can be inhibited without escape after prolonged treatment periods (Hofland & Lamberts 2003). Therefore, desensitization of SSTRs seems to have a minor role, if any, in determining different responsiveness to SRLs.

**Heterogeneity of SSTR expression**

**GH-secreting pituitary adenomas** In contrast to receptor desensitization, variable tumor expression or reduced receptor density of SSTR subtypes can explain the high proportion of patients partially resistant to SRLs (Reubi et al. 1994, 2001, Casarini et al. 2009). Positive correlation exists among SSTR2 mRNA expression, SSTR2 immunohistochemistry positivity, or membrane receptor density with the in vivo GH suppression induced by SRLs (Casarini et al. 2009, Brzana et al. 2013). Therefore, SSTR2 seems to be a predominant receptor in determining the inhibitory effect of octreotide or lanreotide on circulating GH release in acromegalic patients. However, loss of SSTR2 is sometimes encountered in partially SRL-sensitive tumors, where SSTR5 mRNA expression is higher and seems to explain the incomplete resistance despite low SSTR2 mRNA expression (Reubi et al. 1994, Nielsen et al. 2001, Brzana et al. 2013). Interestingly, GH suppression with SSTR2 and SSTR5 bi-specific compound was better achieved (73%) when compared with the use of SSTR2 (32%) or SSTR5 (34%)-selective agonists (Ren et al. 2003b). Additionally, in co-secreting GH and PRL adenomas, PRL secretion is preferentially inhibited by SSTR5-specific ligands (Shimon et al. 1997a,b, Jaquet et al. 2000). Therefore, some adenomas show better response to SSTR2-specific ligands, whereas in others, SSTR5-specific ones are more potent. The additive inhibitory effects on GH release following activation of both SSTR2 and SSTR5 are likely mediated via a functional interaction of both SSTR subtypes (Rocheville et al. 2000, Ren et al. 2003b). Finally, SSTR1 may also play a role in patients poorly sensitive to SRLs. Activation of SSTR1 by a SSTR1-selective ligand decreased GH secretion in in vitro tumor cells derived from patients partially responsive or resistant to SRLs. SSTR1 mRNA levels correlated with the degree of inhibition of GH secretion in vitro (Zatelli et al. 2003, Matrone et al. 2004), as well as SSTR1 mRNA expression was associated with a higher proportion of patients with GH and IGF1 normalization using SRLs (Casarini et al. 2009).

**ACTH-secreting pituitary adenomas** In vitro, and in vivo, studies show that hydrocortisone treatment as well as endogenous hypercortisolemia hampers ACTH suppression by SST or octreotide treatment. By contrast, ACTH secretion was significantly suppressed in normal corticotrope cells cultured in glucocorticoid-free medium as well as in patients with adrenal insufficiency (Lamberts 1988). A plausible explanation is the down-regulation of SSTR2 expression in corticotrope adenomas by glucocorticoids (Fig. 2). Interestingly, after achieving eucortisolemia with pasireotide in patients with CD, cortisol-mediated SSTR2 down-regulation in corticotrope adenomas was reversible at the mRNA level, but not at the protein level. It is not clear if sustained eucortisolemia induced by medical therapy can induce re-expression of functional SSTR2 protein (van der Pas et al. 2013).

**Conclusions**

SST and SSTR are broadly expressed in humans, including normal pituitary tissue and pituitary adenomas. Thus, SRLs have a therapeutic value in several pituitary disorders; they are the mainstay of medical therapy in acromegaly and have also been recently approved for CD patients after failure of surgery. However, a large proportion of patients are at least ‘partially resistant’ to treatment in both diseases. The term SRL ‘resistance’ per se remains elusive. The exact mechanism is yet unclear, and it is likely that multiple factors are involved.

Clinical predictors of response in patients with acromegaly include gender, age, initial GH and IGF1 levels, and possibly tumor size. Several molecular predictors such as reduced sensitivity of SSTRs, SST receptor internalization, heterogeneity of SSTR expression, and signaling pathway disruption seem to also play an important role.

Development of novel multiligand SRLs, chimeric molecules, and combination therapies could further improve the response rates and/or decrease the incidence of adverse events in acromegaly, CD, and other hypersecretory or non-functioning pituitary tumors.
We recommend to include both biochemical and tumor effects to define the concept of SRL resistance in a better way. Further understanding of the clinical, molecular, and histopathological predictors of resistance will help optimize individualized treatment for each patient.

Declaration of interest
D C-R declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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Cushing’s disease.

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