



“The Cushing Game”: The Molecular Basis of Combined Therapy

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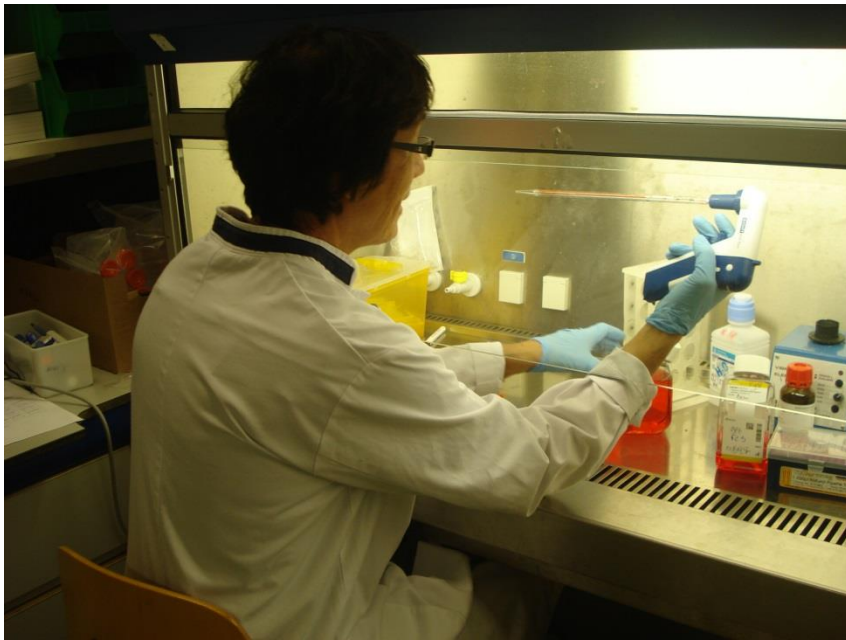
Erasmus Medical Center - old



Erasmus Medical Center - new



Pituitary tumor cell culture



Pituitary tumor cell culture



Outline

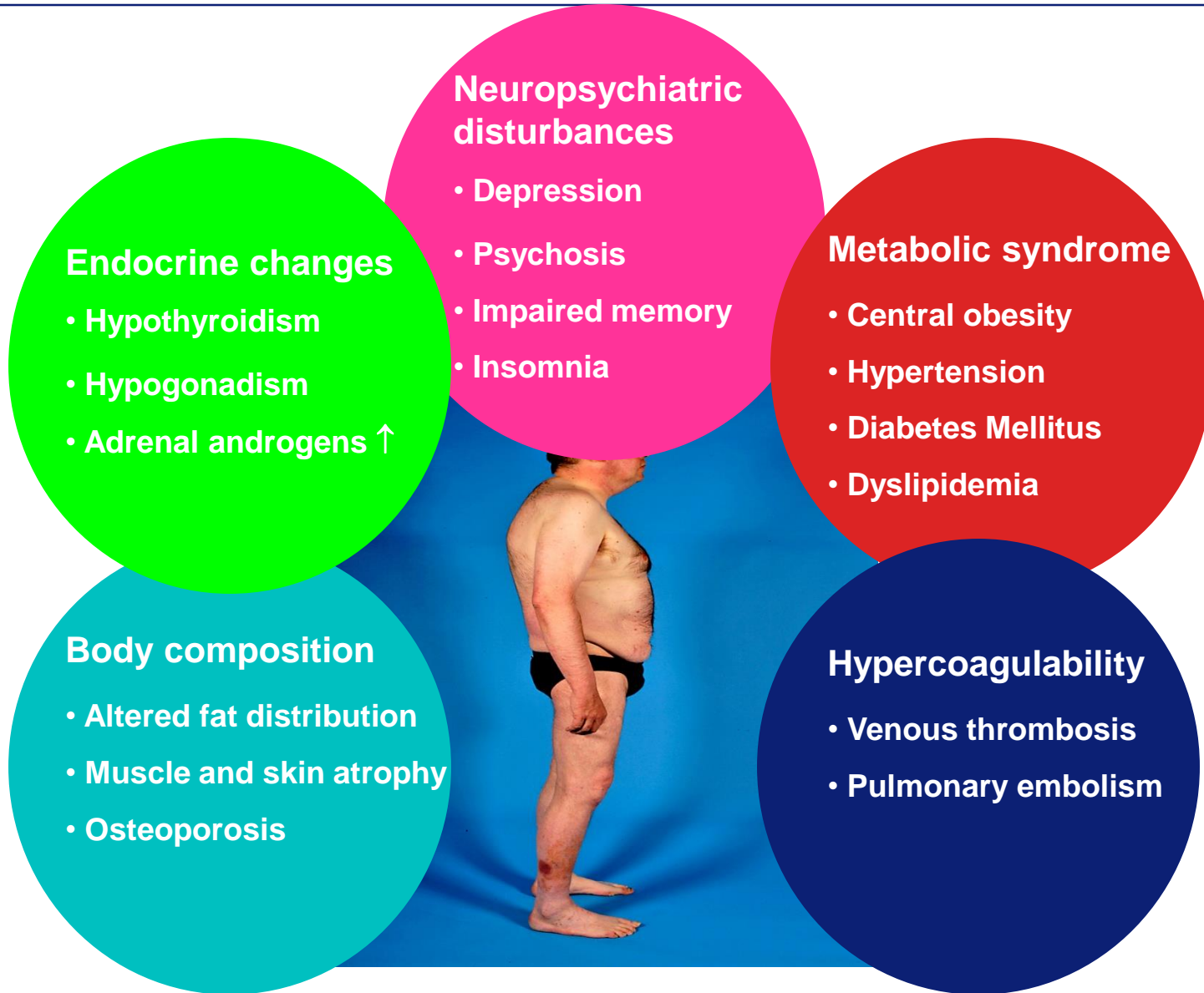
- **Introduction**
- **Medical treatment of CD and rationale of combination therapy**
- **Targets for and mechanisms of combined medical treatment**
- **Conclusion**

Cushing's syndrome

- **ACTH-dependent:**
 - **pituitary adenoma**
 - **ectopic ACTH production**

- **ACTH-independent:**
 - **adrenal adenoma**
 - **bilateral adrenal hyperplasia**
 - **adrenal carcinoma**

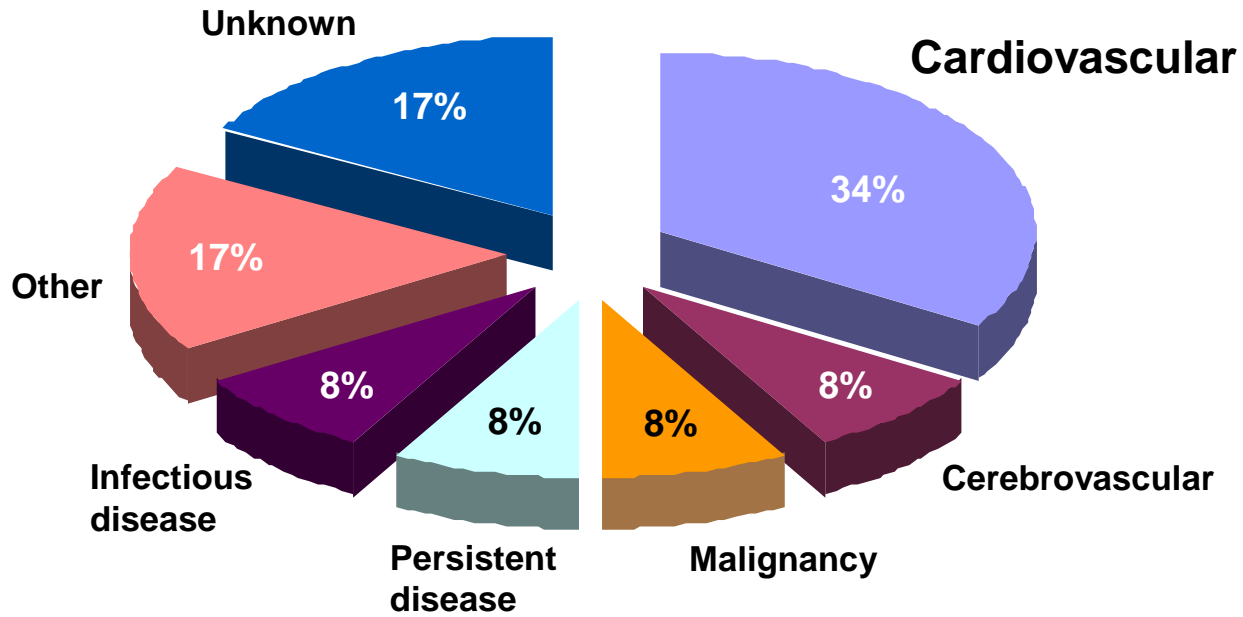
Cushing's disease: morbidity



Mortality in Cushing's disease

First author, year	SMR cure	SMR persistent CD
• Lindholm, 2001	0.31	5.1
• Hammer, 2004	1.18	2.8
• Dekkers, 2007	1.8	4.38
• Clayton, 2011	3.3	16
• Hassan-Smith, 2012	2.47	4.12
• Ntali, 2013	10.8	9.9

Cardiovascular disease is a leading cause of death



Reversibility of co-morbidity

Feature	Prevalence	Persistence after remission
Overweight (BMI 25–30)	21–48%	33 %
Obesity (BMI >30)	32–41%	40 %
Impaired glucose tolerance	21–64 %	60 %
Diabetes mellitus	20–47 %	40–60 %
Hypertension	55–85 %	44–75 %

Treatment of Cushing's disease

- Transsphenoidal adenomectomy is the first-line treatment of Cushing's disease (CD)
- Remission rates after surgery vary between 60-90 %, but up to 25 % of patients develops recurrent CD
- Options after surgical failure:
 - **second surgery**
 - **radiotherapy**
 - **bilateral adrenalectomy**
 - **medical therapy**

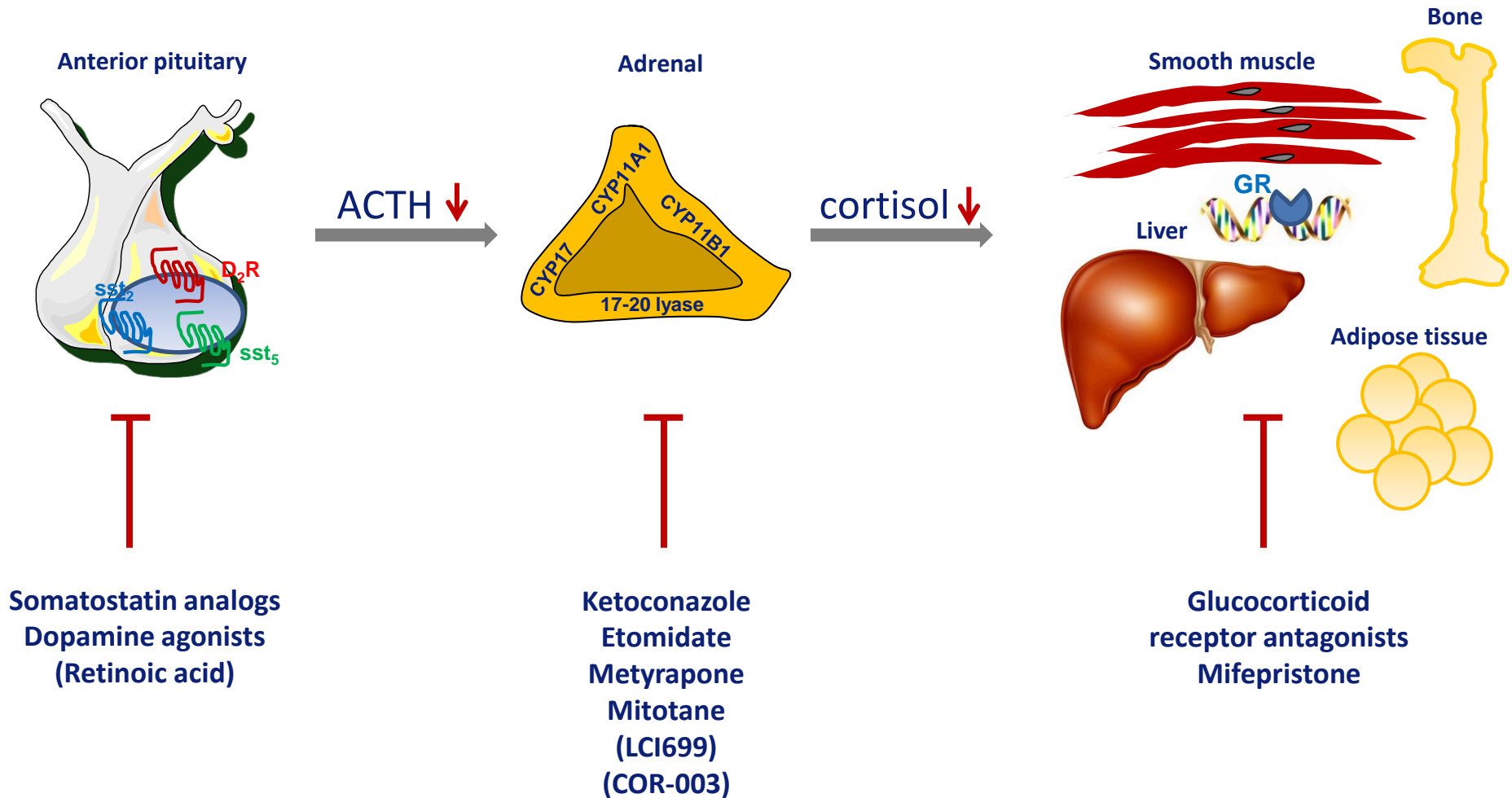
Indications for medical therapy

- **Cushing's disease:**
 - pretreatment before surgery
 - after unsuccessful surgery and RT
 - primary medical therapy

- **Severe complications of hypercortisolism**

- **Inoperable/metastasized tumors:**
 - ectopic ACTH syndrome
 - cortisol producing adrenal carcinoma

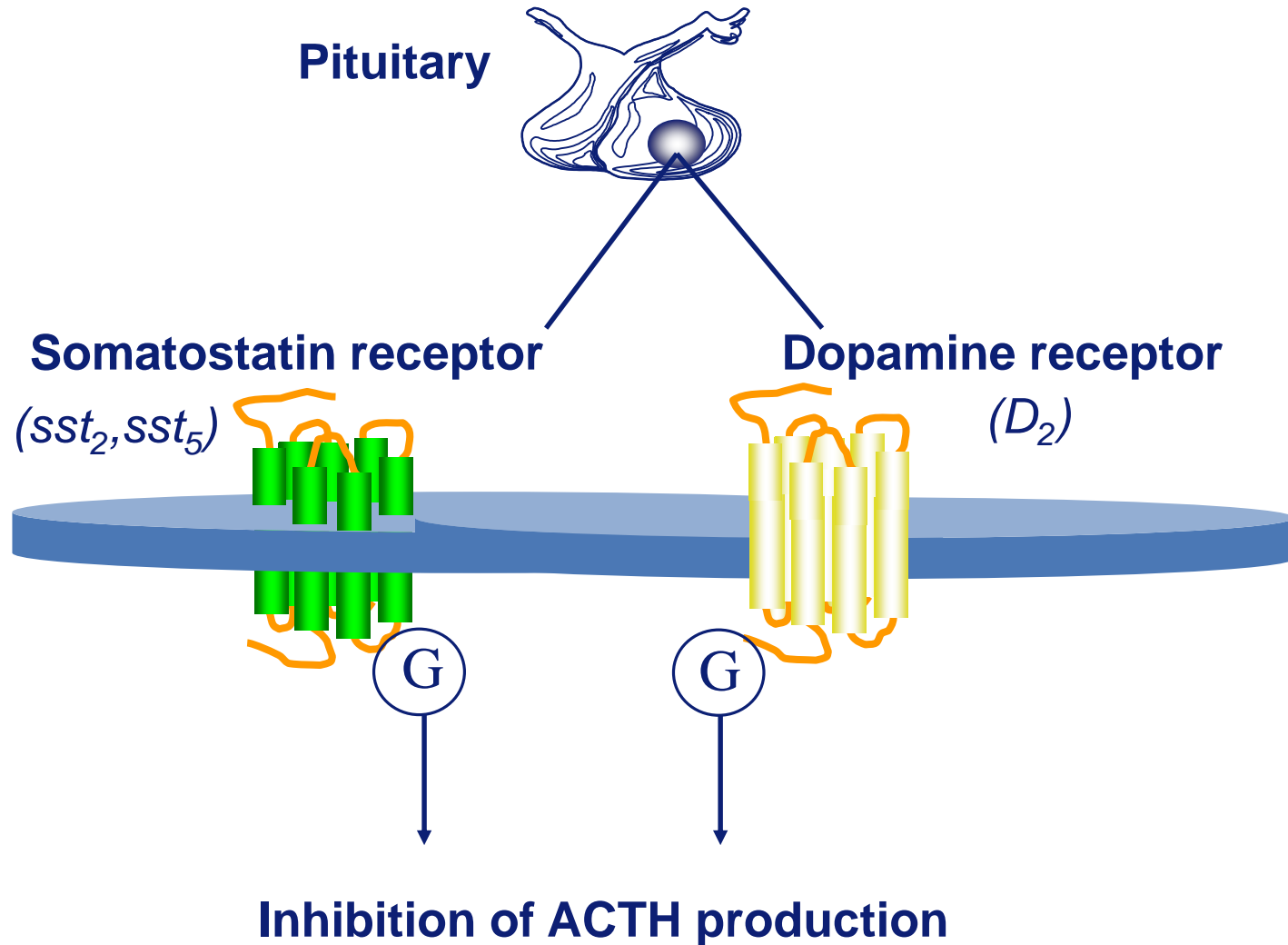
Drug targets in Cushing's disease



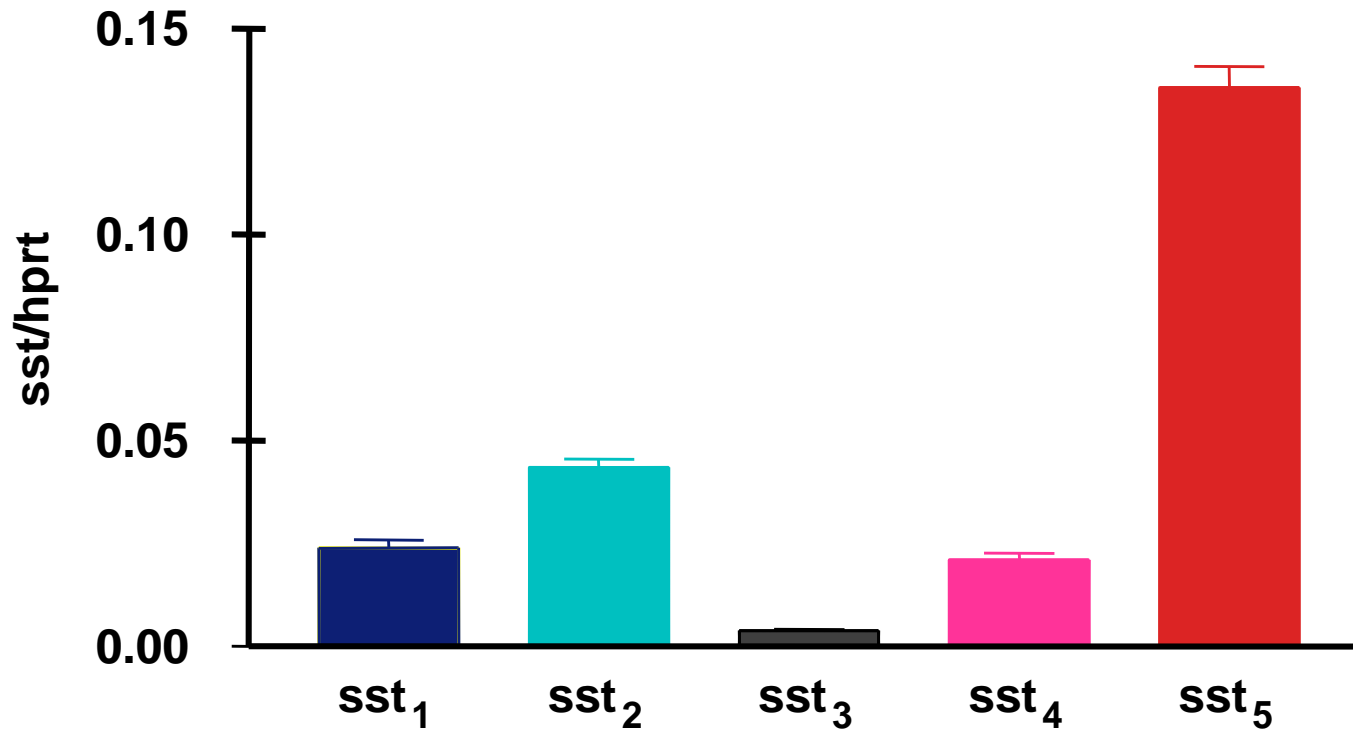
Combination therapy

- **Indicated:**
 - **in patients with moderate to severe hypercortisolism**
 - **in patients with acute complications of hypercortisolism**
- **Lower dosages may cause less side effects**
- **Combining compounds may have synergistic effects**

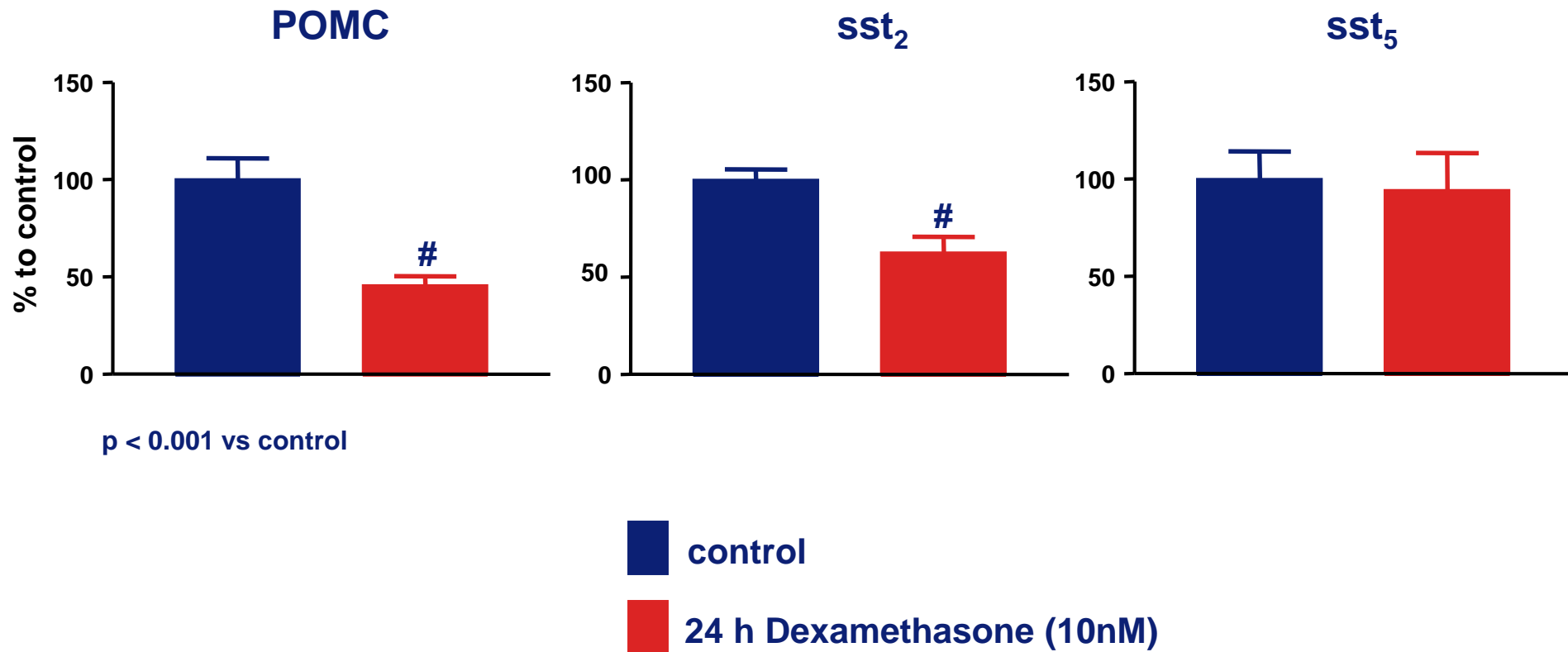
Somatostatin and dopamine receptors



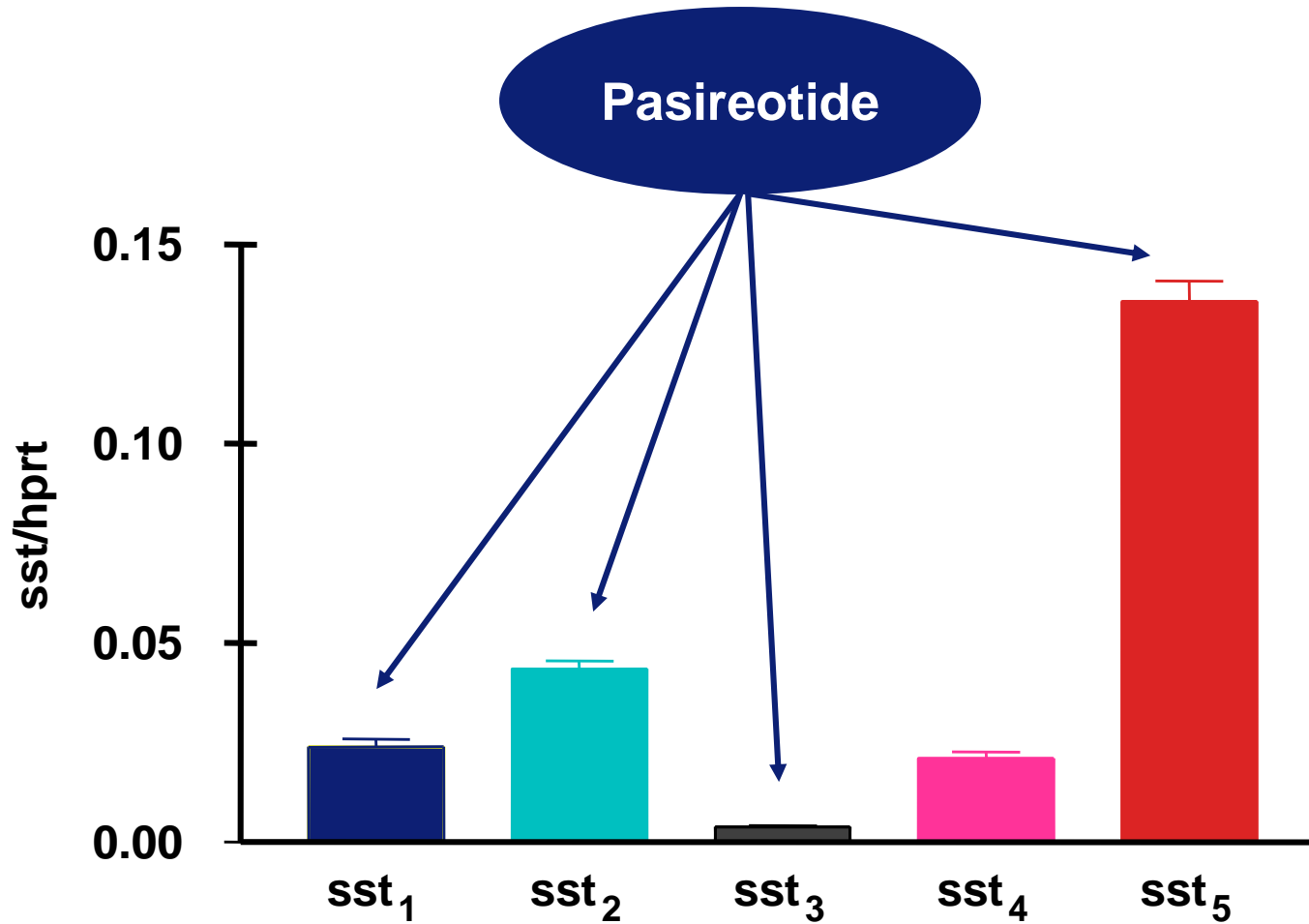
Somatostatin receptor subtype expression in corticotroph adenomas (n=30)



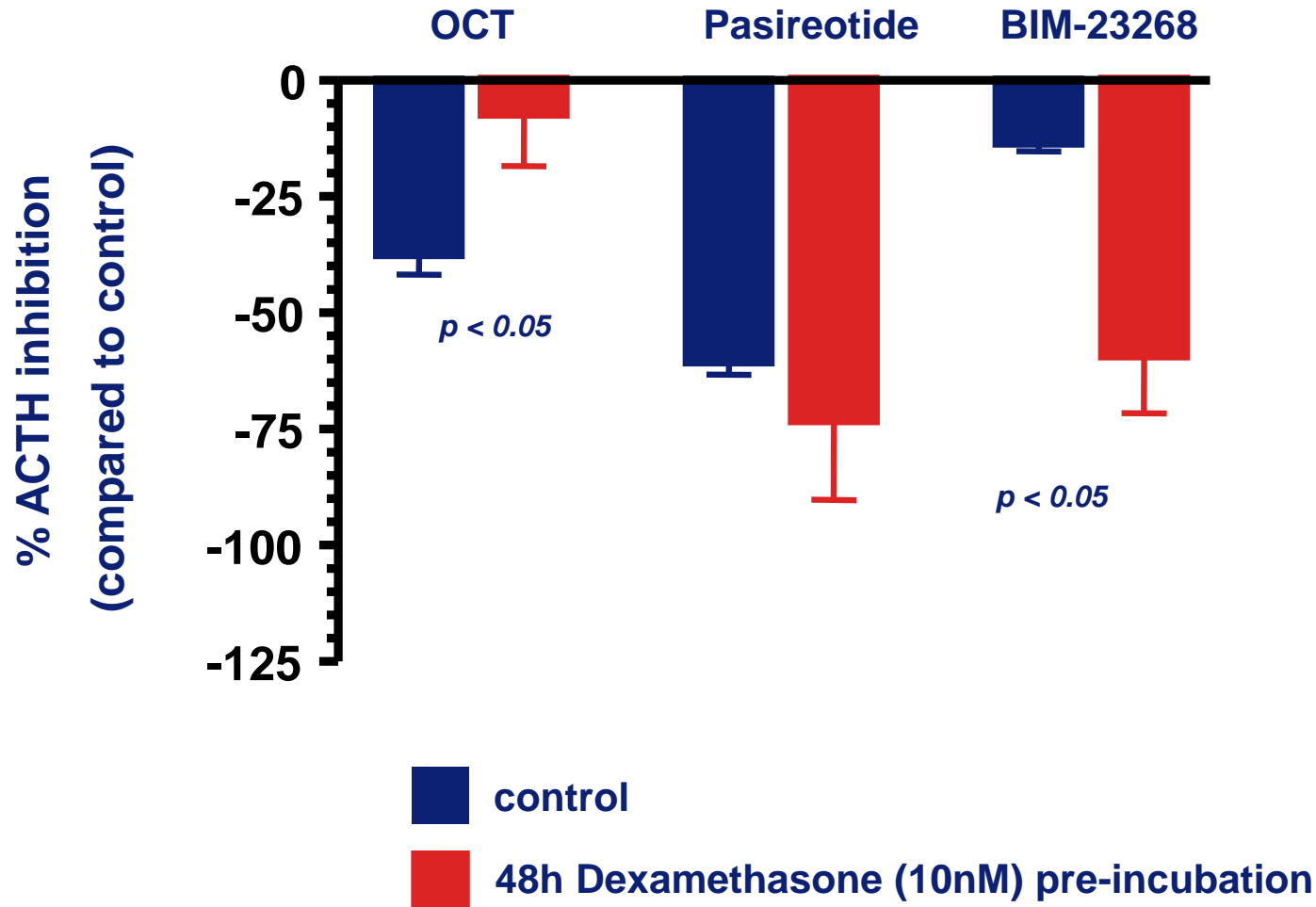
Effect of glucocorticoids on sst mRNA expression levels in AtT-20 cells



Pasireotide: universal somatostatin analogue

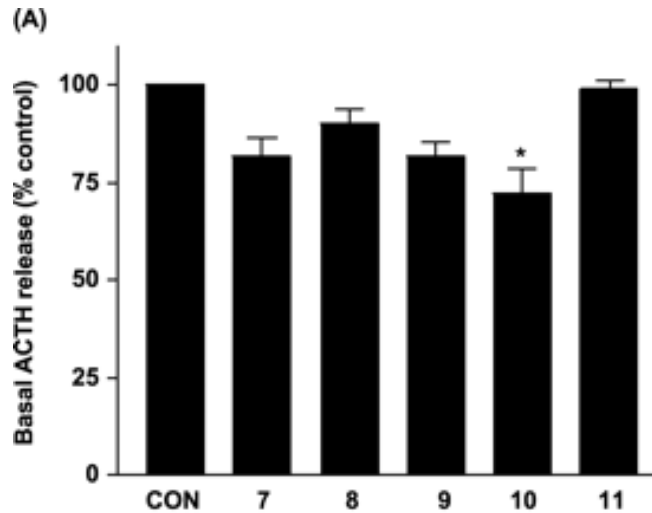


Effects of glucocorticoids on ss-analog mediated inhibition of ACTH secretion (AtT20 cells)

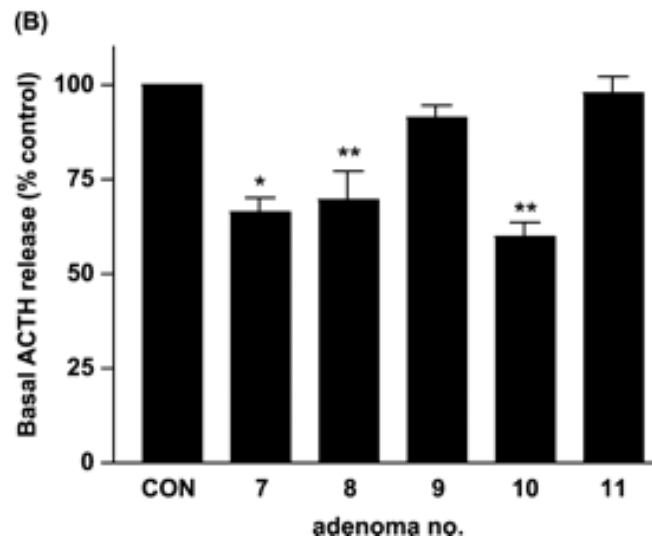


Effects of octreotide and pasireotide on ACTH secretion by human corticotroph adenomas

Octreotide



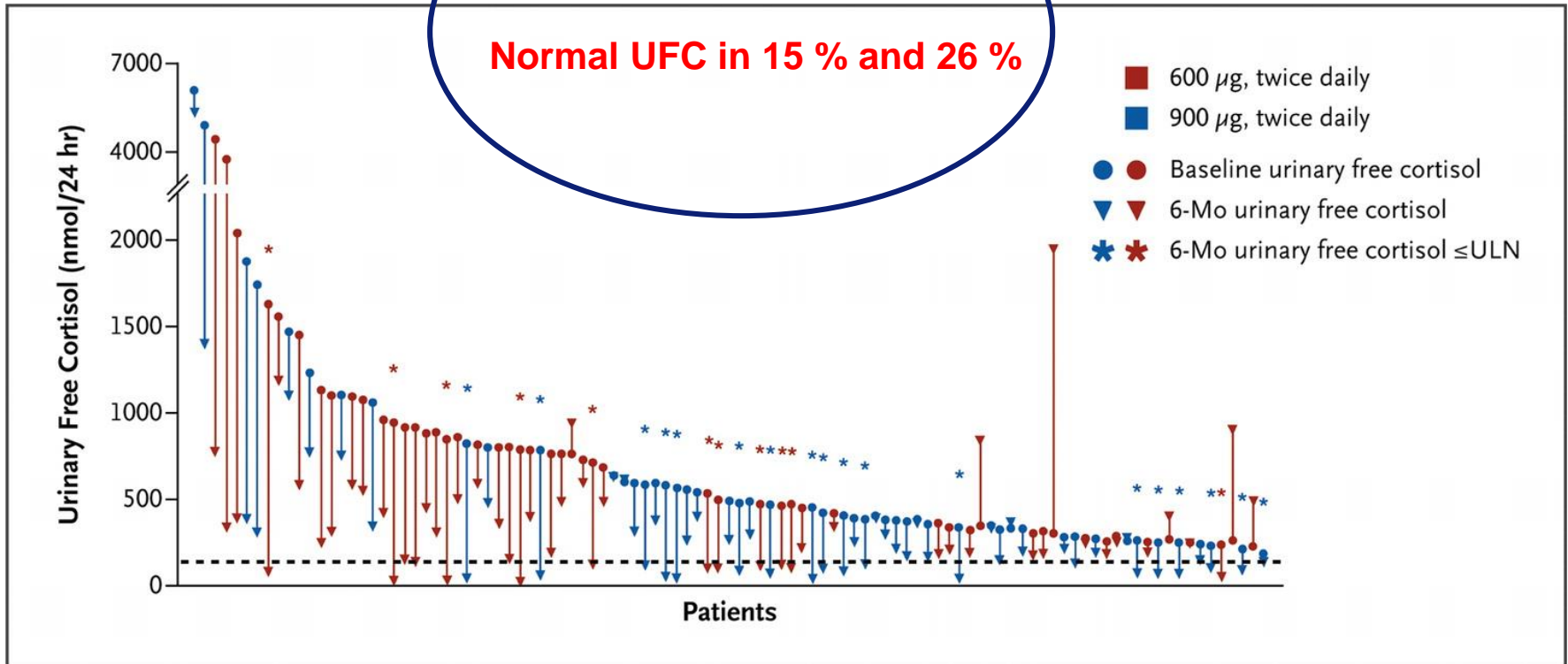
Pasireotide



Phase III trial with pasireotide (n=162)

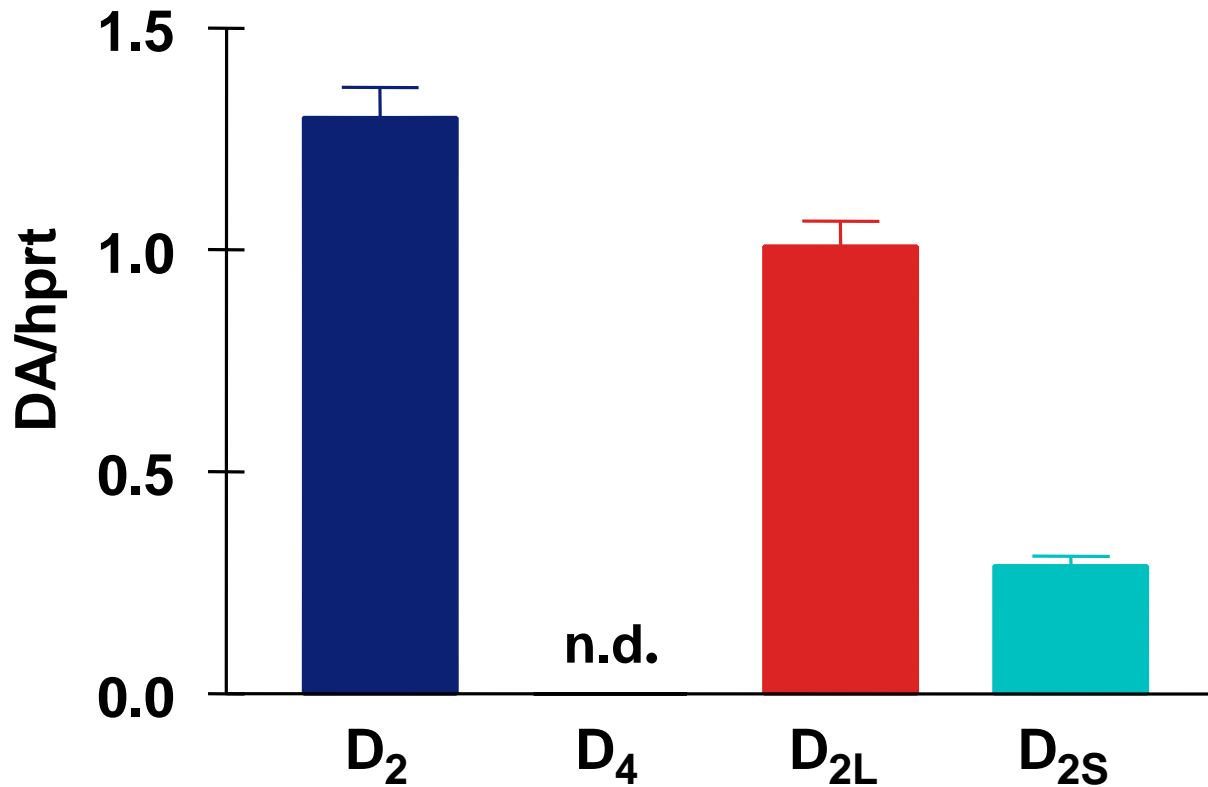
Average UFC ↓ 50 %

Normal UFC in 15 % and 26 %

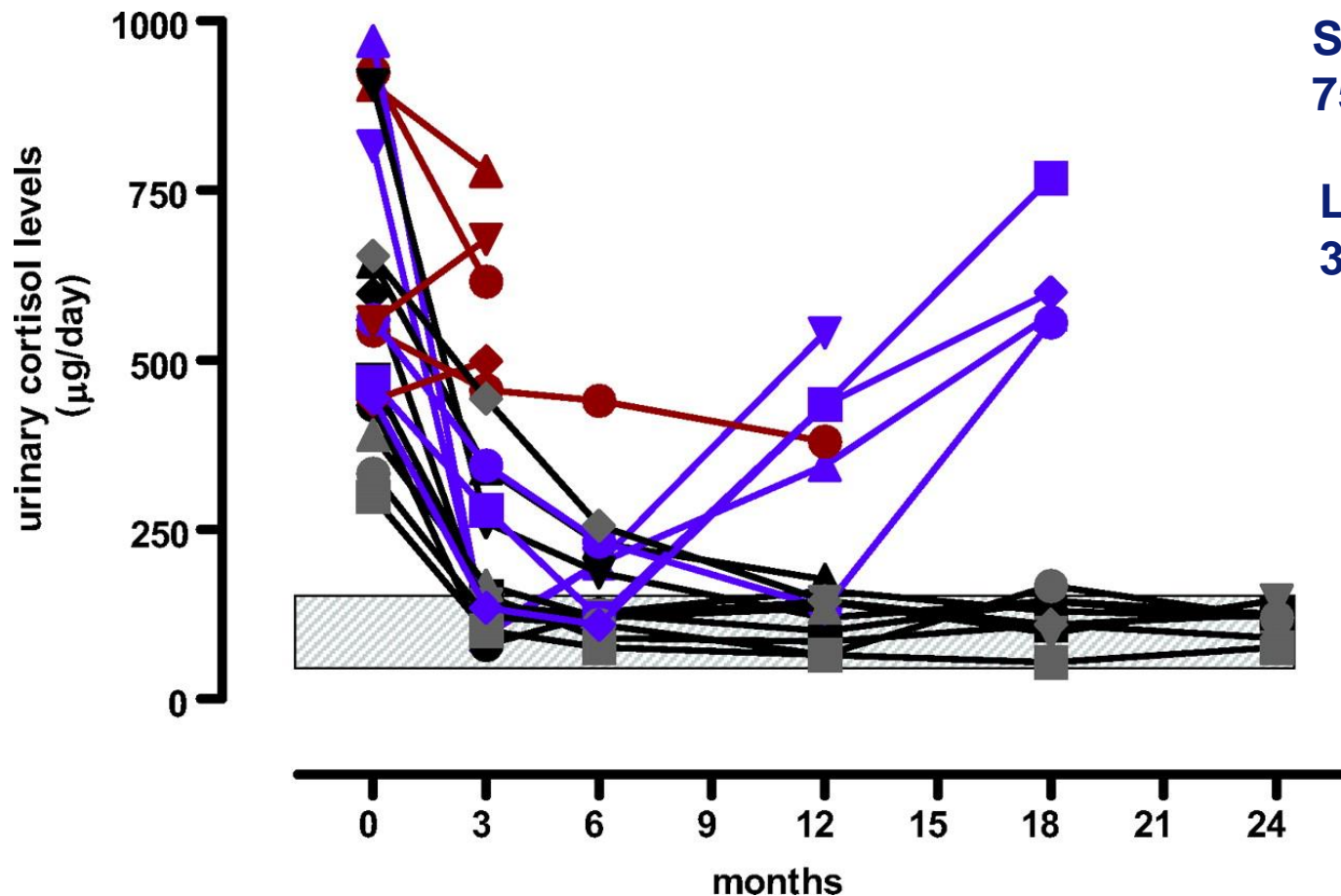


Dopamine receptor

Dopamine receptor subtype 2 expression in corticotroph adenomas (n=30)



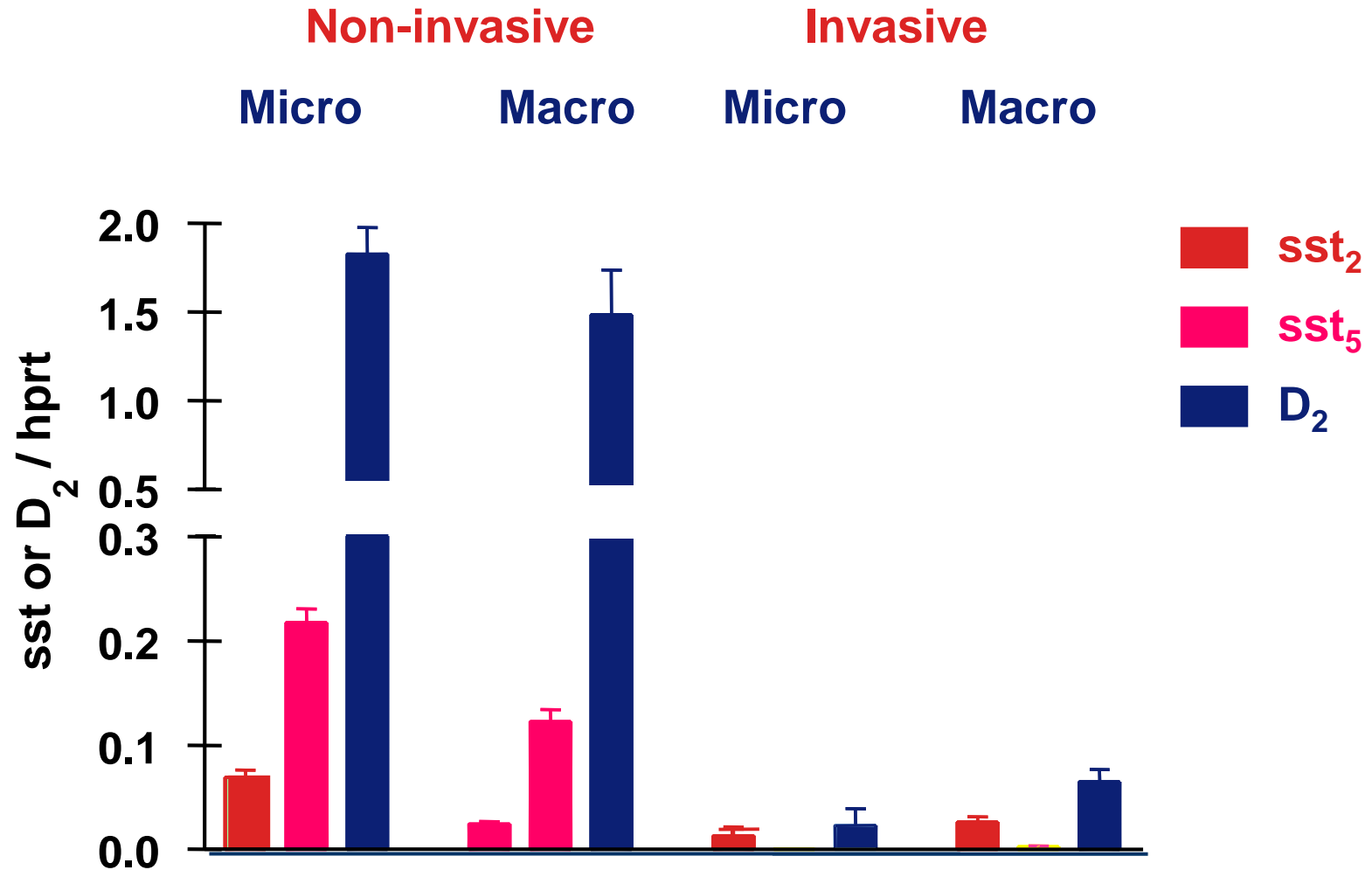
Effects of cabergoline on UFC in Cushing's disease



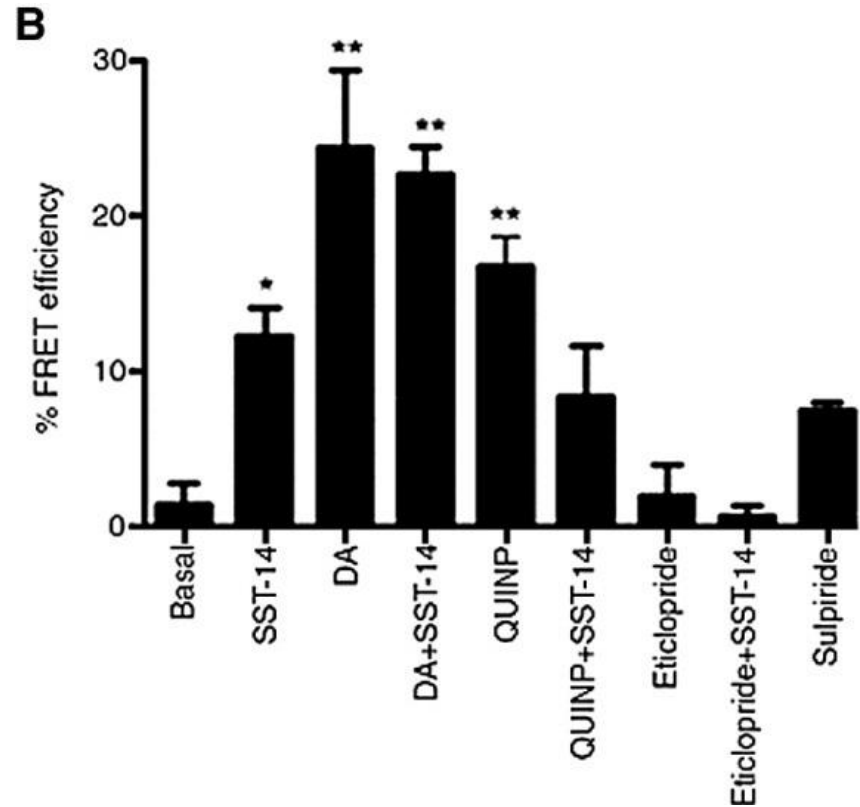
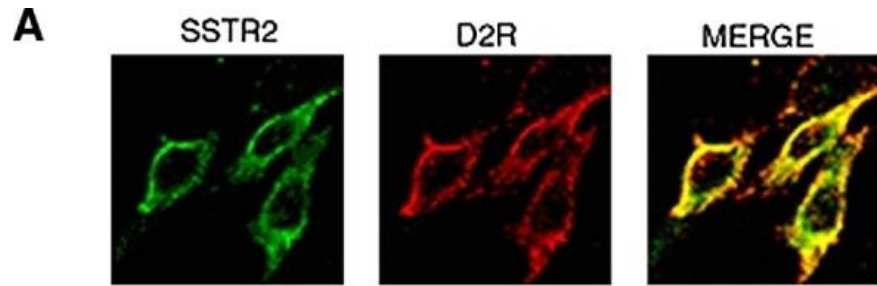
Short-term response:
75 %

Long-term response:
35 %

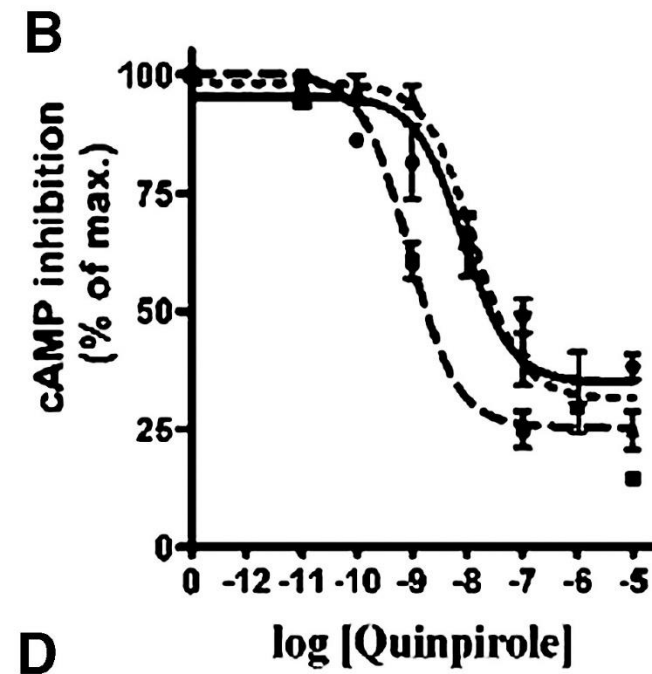
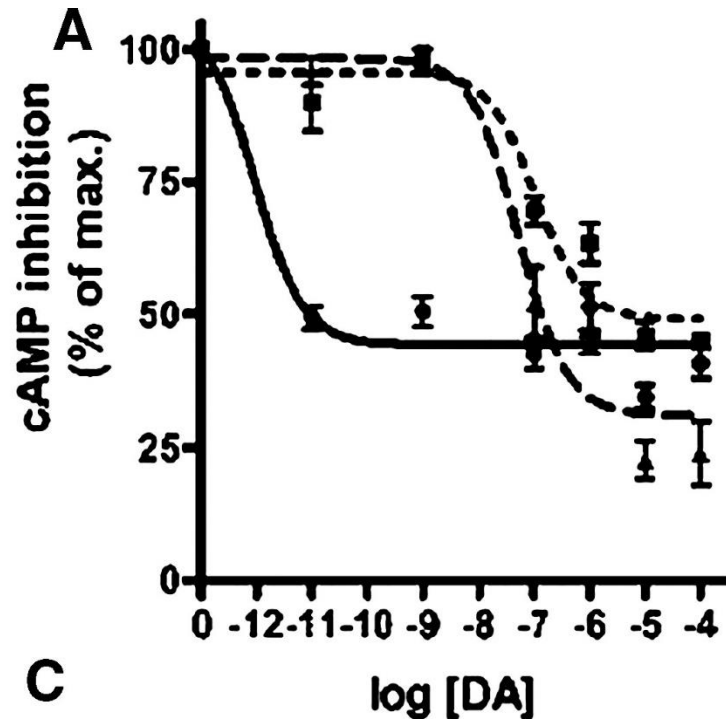
Sst₂, sst₅ and D₂ receptor expression in corticotroph adenomas



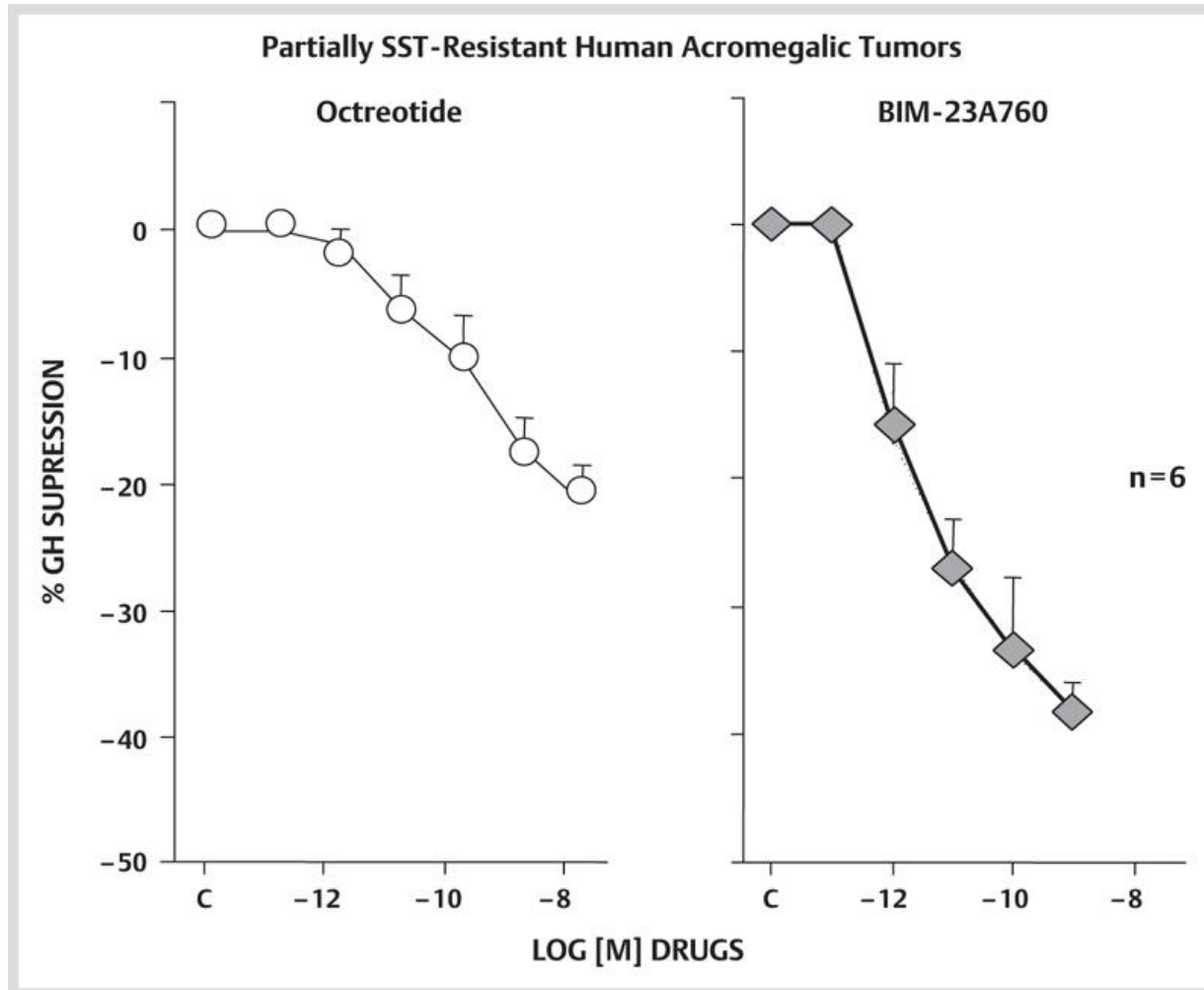
Hetero-oligomerization of human sst₂ and D2



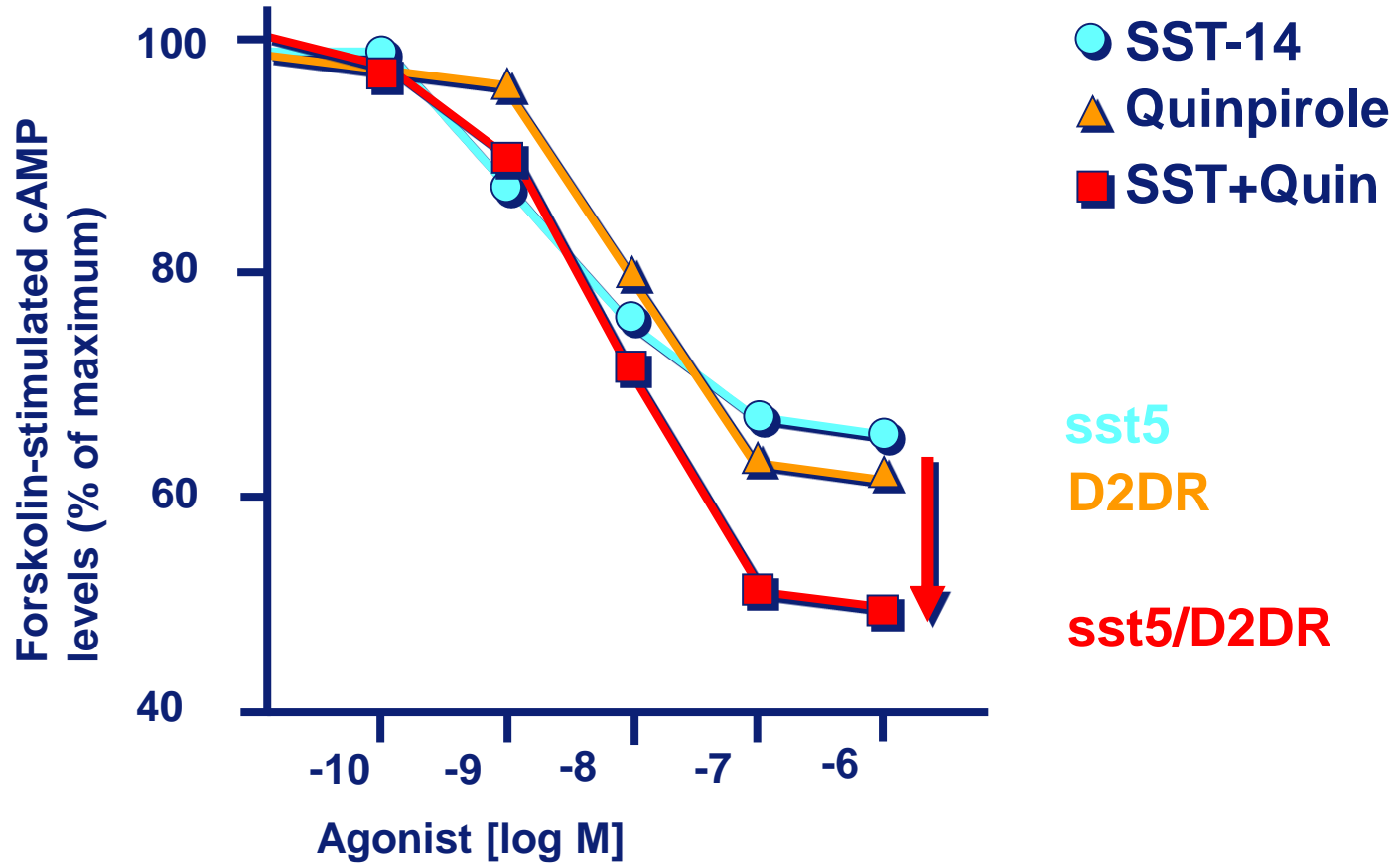
Inhibition of cAMP formation via D2 and sst₂



Somatostatin-dopamine chimeric compound



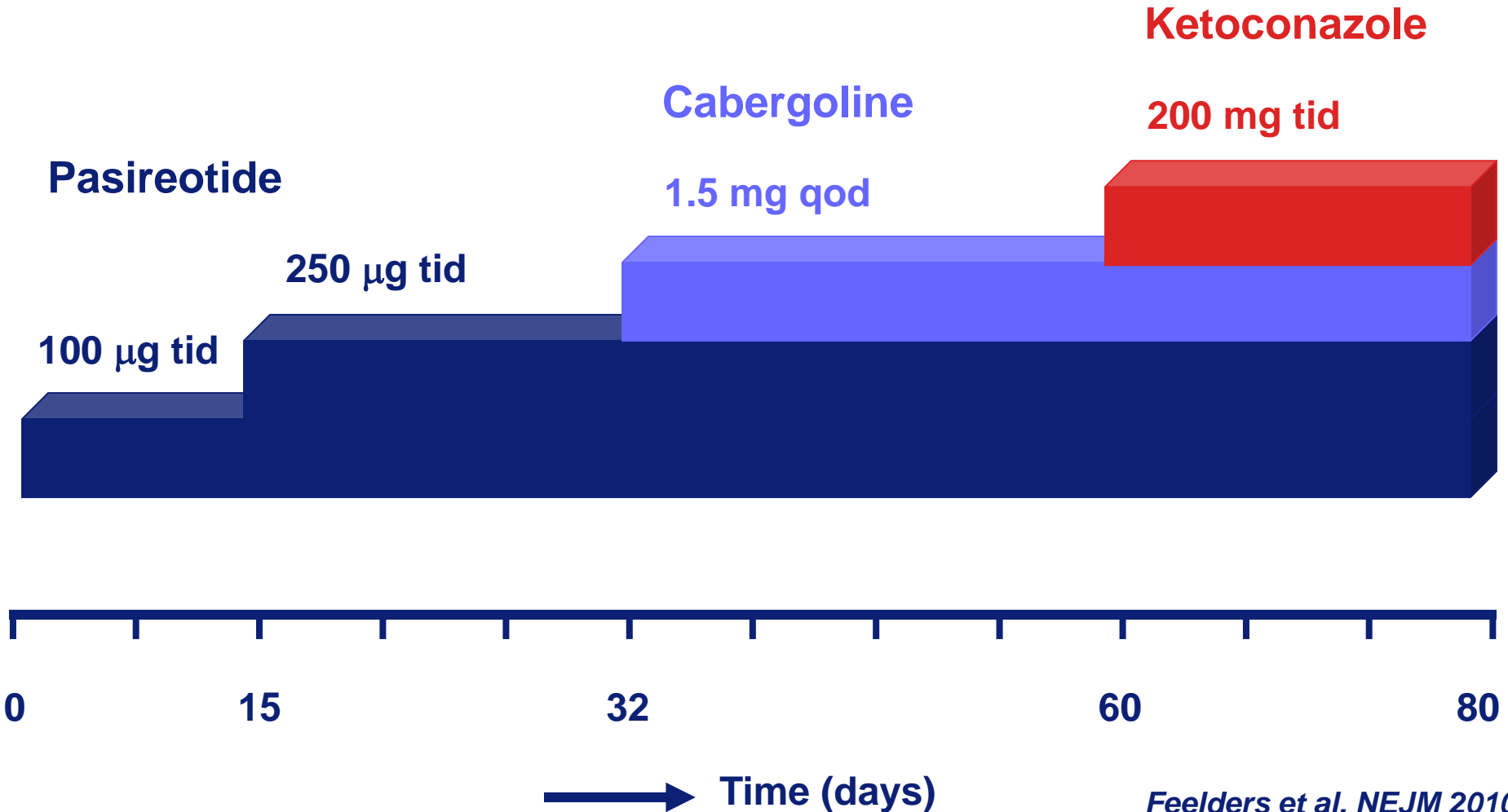
Enhanced functional activity of D2DR-sst₅ heterodimers



Combination therapy

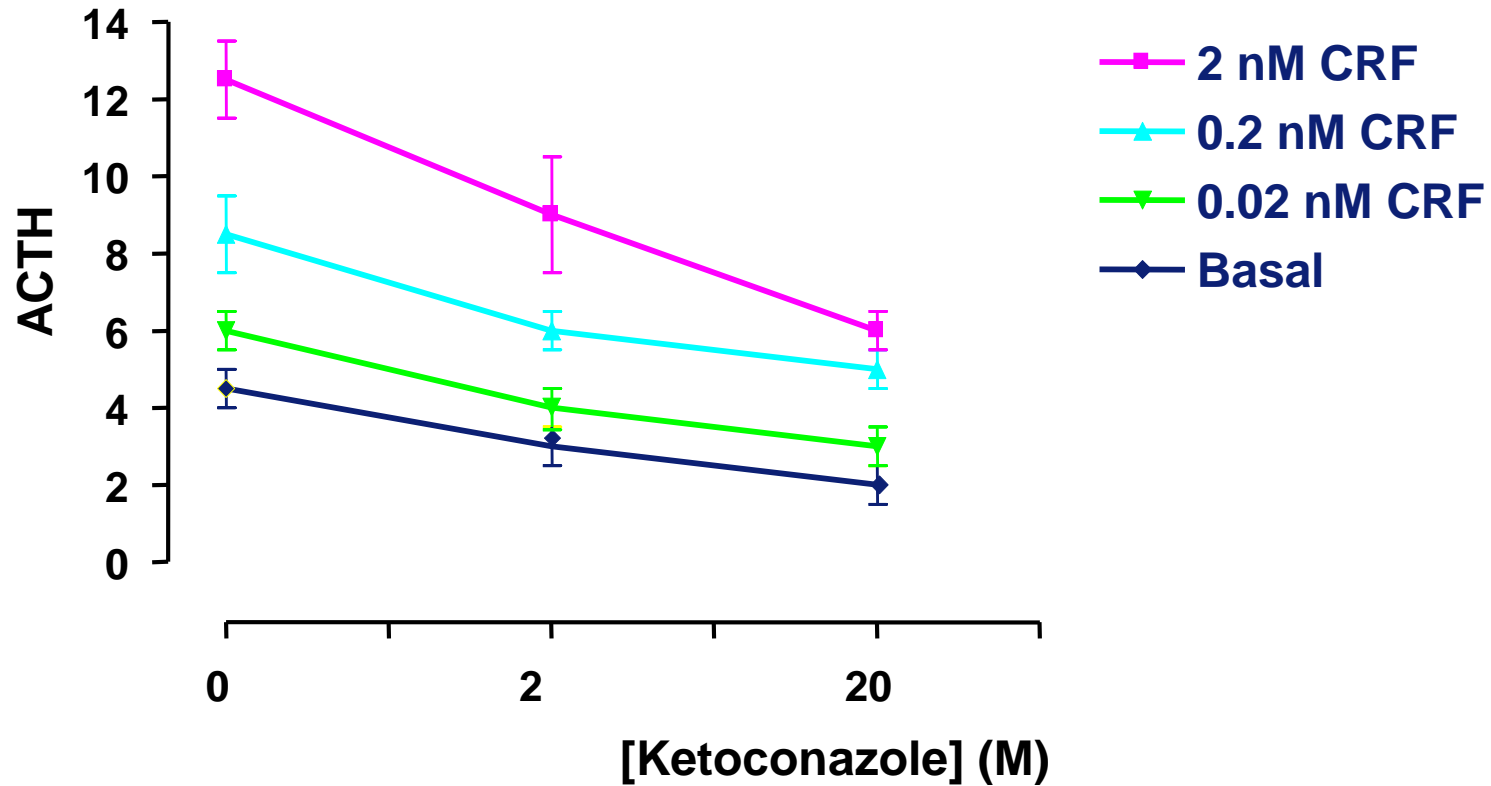
- **Pasireotide and cabergoline alone can induce biochemical remission in \pm 25-30 % of CD patients**
- **Combined targeting of sst and Da receptors may result in additive or synergistic effects on ACTH secretion by corticotroph tumor cells**

Pasireotide mono- or combination therapy (n=17)



Neuromodulatory effects of ketoconazole

Dose-dependent inhibitory effect of ketoconazole on basal and CRF-induced ACTH release by rat anterior pituitary cells



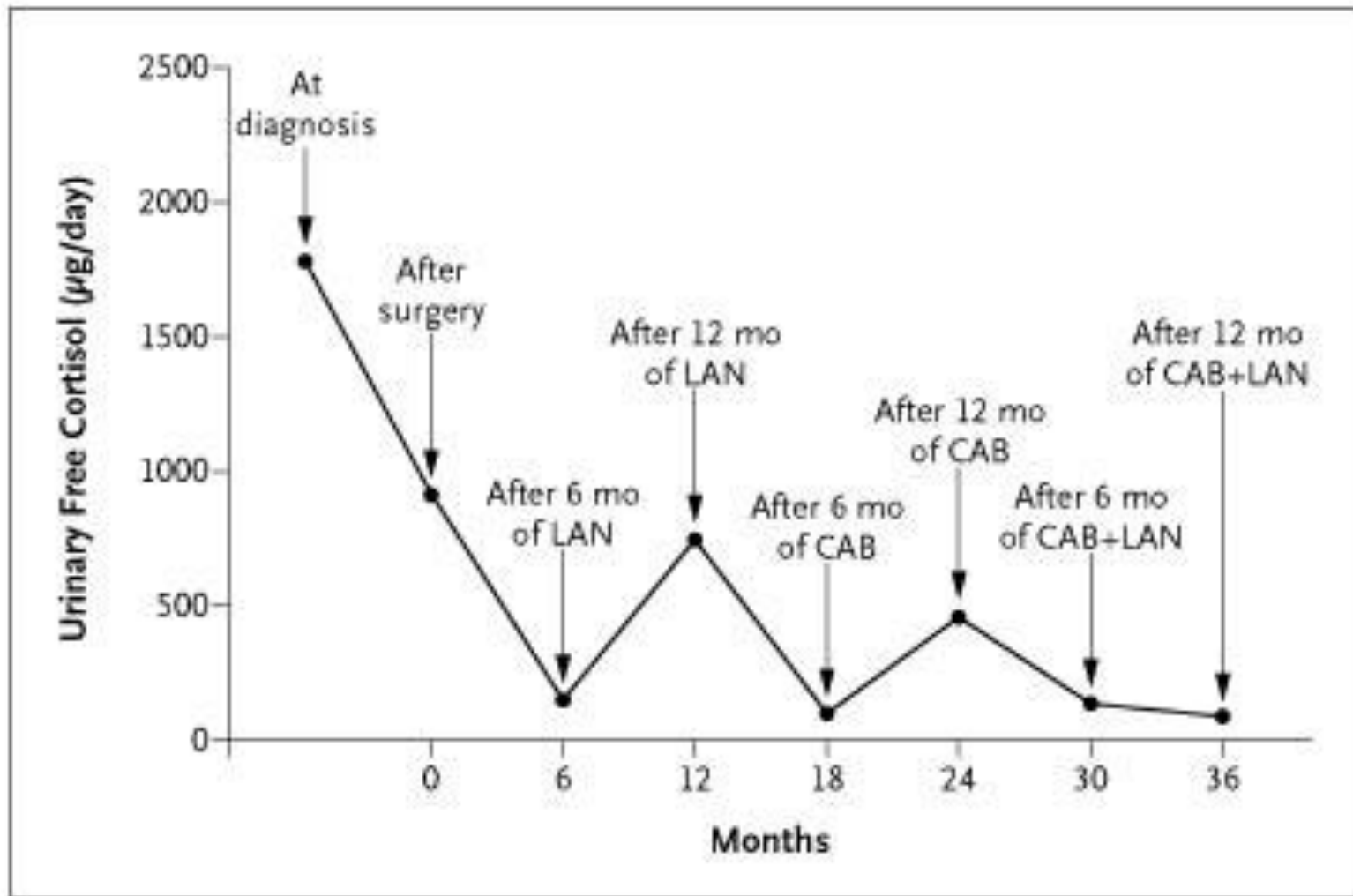
Neuromodulatory effects of ketoconazole *in vivo* ?

- **ACTH response to CRH is unchanged or enhanced after ketoconazole treatment (Loli et al. 1986, Boscaro et al. 1987)**

- **No compensatory rise in ACTH levels upon prolonged treatment with ketoconazole (Loli et al. 1986, Sonino et al. 1991)**

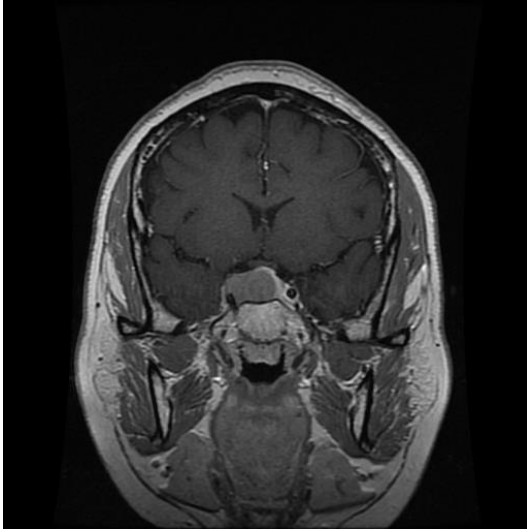
Sst₂, sst₅ and D₂ receptor expression in neuroendocrine tumors

Cabergoline and lanreotide for ectopic Cushing's syndrome



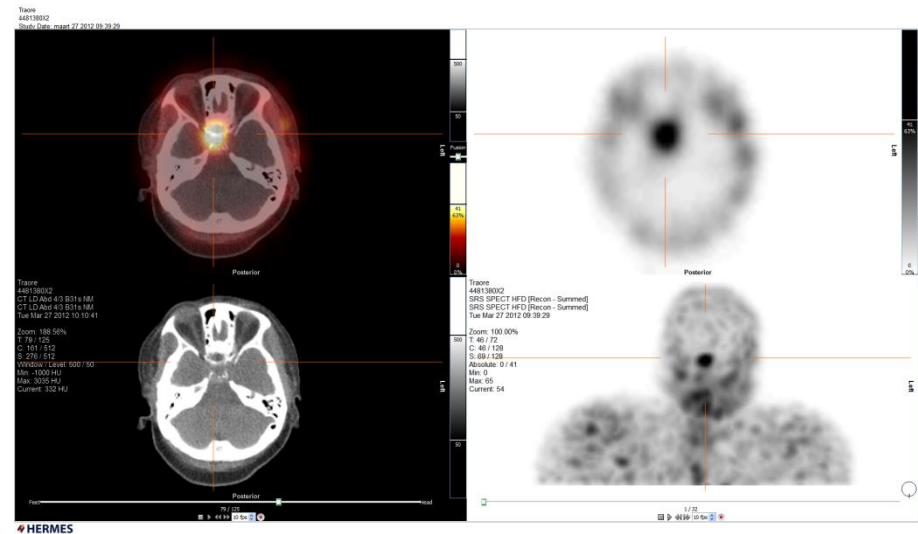
Is cortisol-mediated ssr_2 down-regulation reversible ?

Patient with a corticotroph macroadenoma

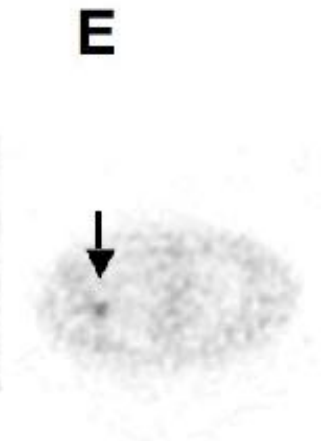
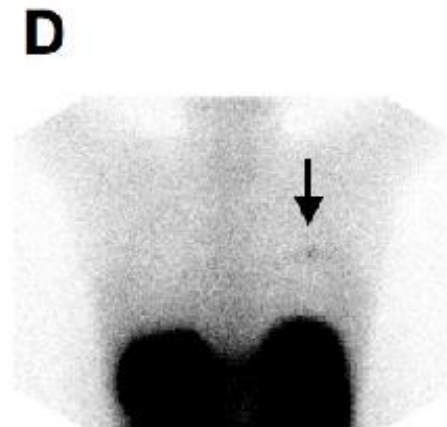
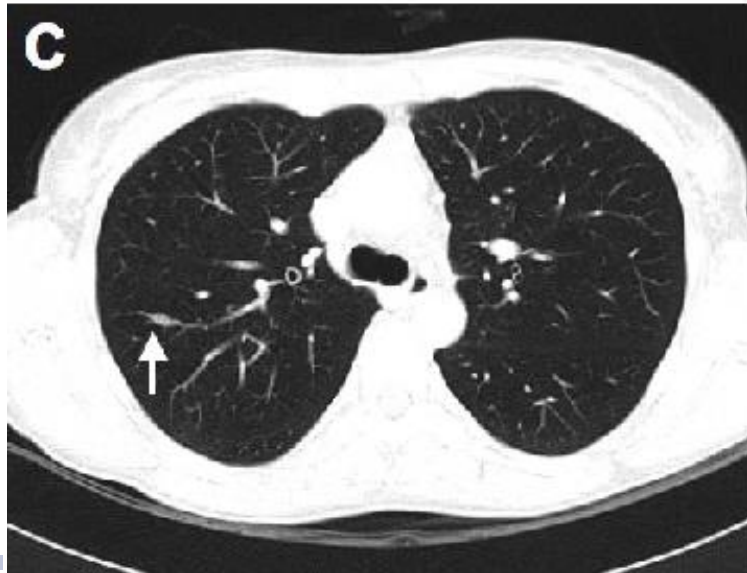
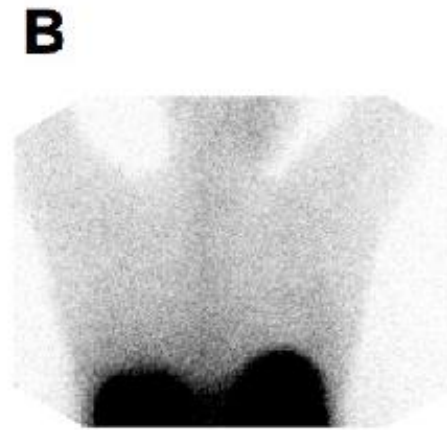
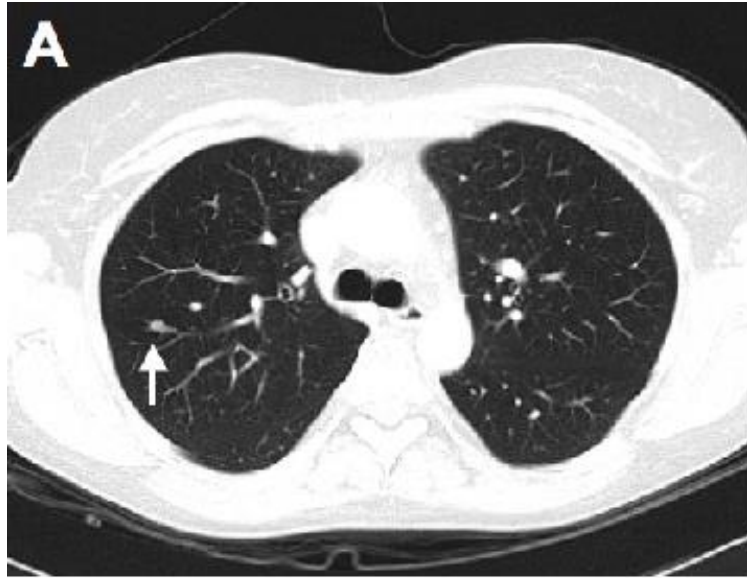


Female, 27 years

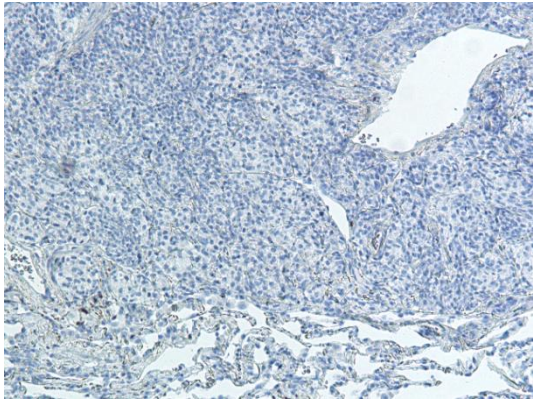
- ACTH-producing macroadenoma
- Initial SMS-scan negative
- SMS-scan positive after 4 months treatment with ketoconazole



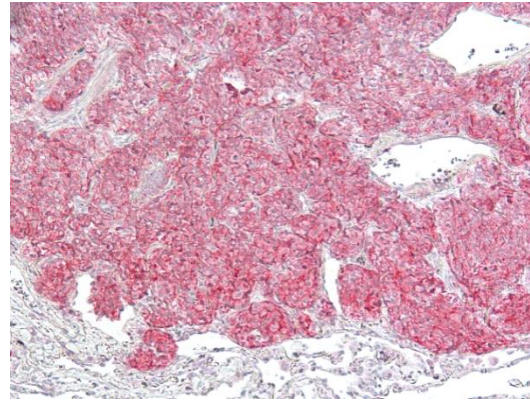
CT and octreoscan before and after treatment



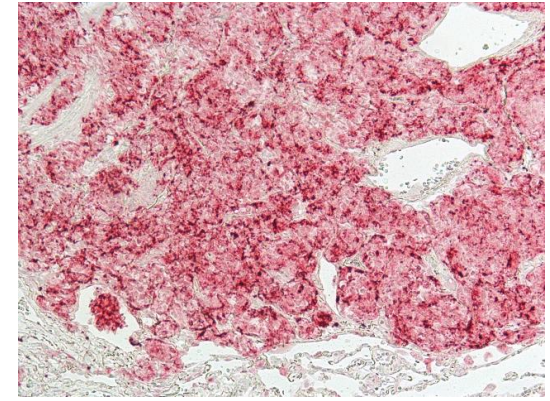
Immunohistochemistry for the sst₂ and D₂ receptor in patient's bronchial carcinoid tissue



Negative control



Sst₂ staining

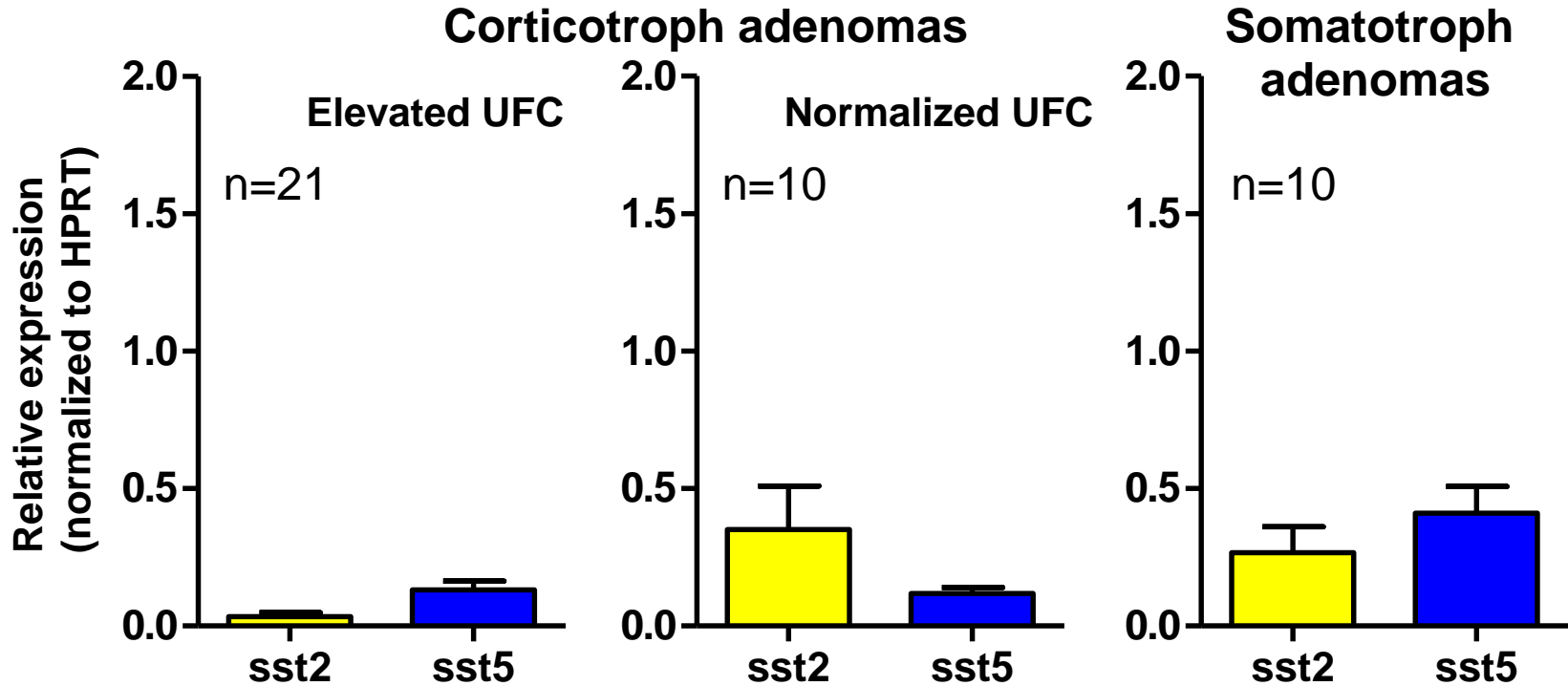


D₂ staining

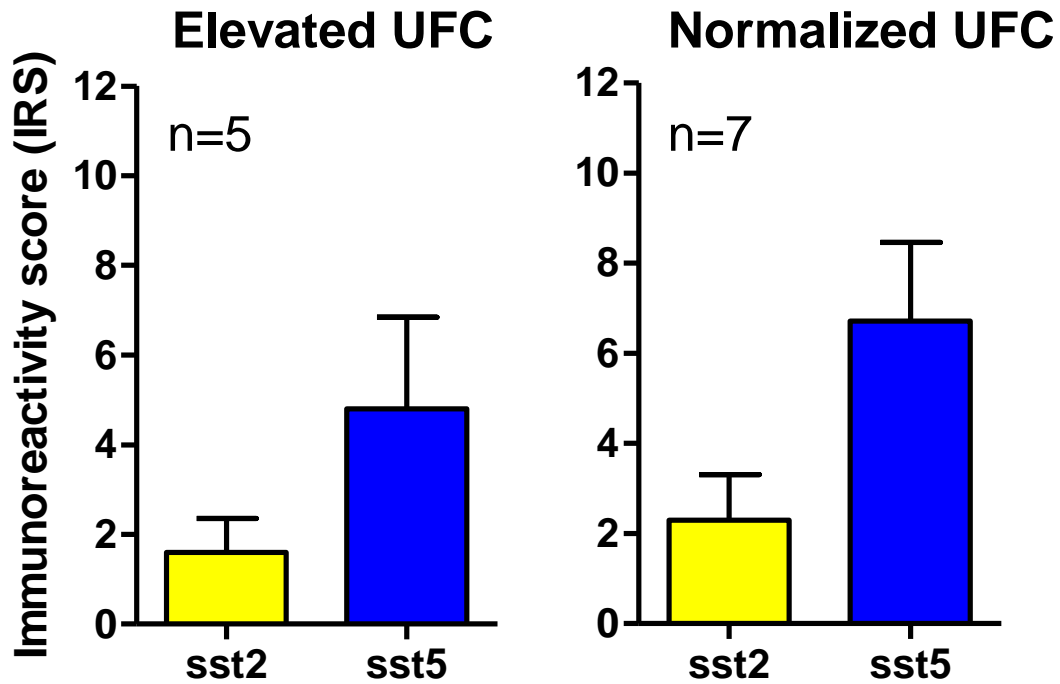
Sst₂ expression in CD after medical treatment

- Evaluation of sst₂ and sst₅ expression in adenomas of:
 - **21 patients with elevated cortisol production preoperatively**
 - **11 patients with normalized cortisol production preoperatively**
- Mean duration of normocortisolism: 10.3 weeks
- Compared with GH-producing pituitary adenomas (n=10).

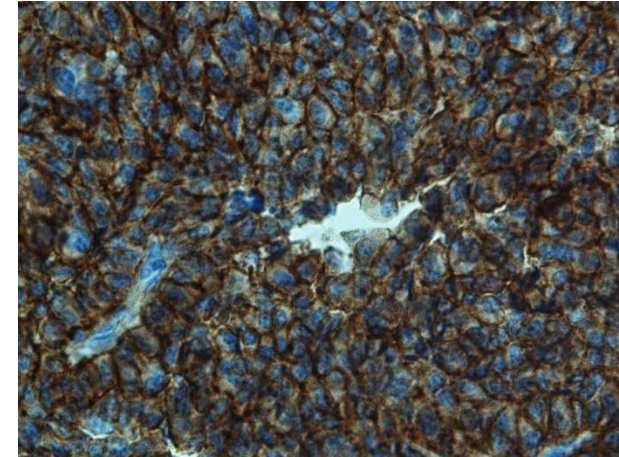
Sst₂ and sst₅ mRNA expression



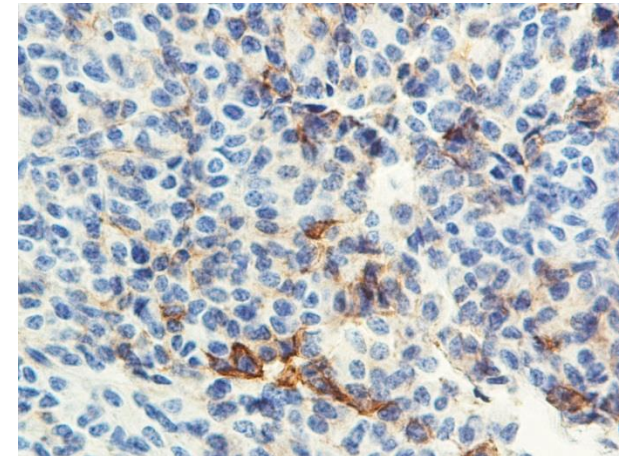
Sst₂ and sst₅ protein expression



sst₅



sst₂



- **Medical treatment of CD is indicated when surgery is not successful or not feasible and should aim to induce complete biochemical remission**
- **In patients with moderate to severe hypercortisolism combination therapy is often needed to control cortisol excess**
- **Somatostatin and dopamine receptors are co-expressed by the majority of corticotroph adenomas**
- **Combined targeting of somatostatin and dopamine receptors may have synergistic effects in suppressing ACTH production**

- Cortisol-mediated sst_2 down-regulation may be reversed via:
 - cortisol-lowering therapy
 - glucocorticoid receptor antagonizing therapy
- Sequential or simultaneous treatment with adrenal blocking drugs (or mifepristone) may enhance the ACTH-lowering potential of pasireotide or even of sst_2 -preferring somatostatin analogs
- Future studies should explore the optimal order and combination of medical treatment modalities for CD

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