# Resistance to cabergoline treatment in human primary non-functioning pituitary tumour cells

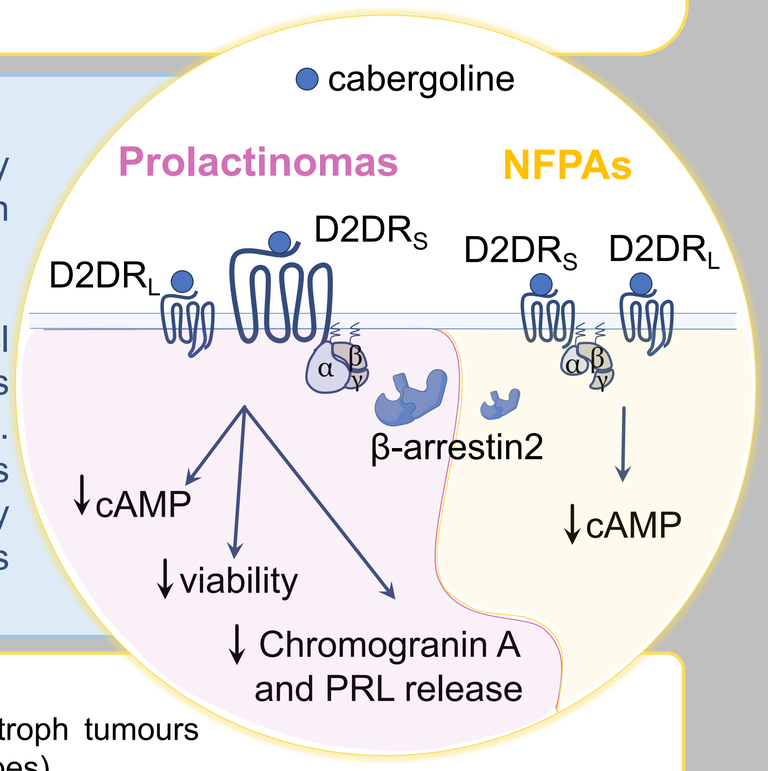
**Begalli F[1], Tatsuya K[2], Suleyman O[1], Magid K[1], Rice T[1], Collier D[1], Dorward N[3], Grieve J[3], Mendoza N[4], Nair R[4], Kolias A[3], Khan D[3], Marcus HJ[3], Botta J[1], McCormick PJ[1], Shinozaki K[2], Korbonits M[1]**

[1]William Harvey Research Institute, Barts&London School of Medicine, Queen Mary University of London, [2]ONO Pharmaceutical Co., Ltd, Osaka, Japan, [3]UCL Queen Square Institute of Neurology, London, UK, [4]Charing Cross Hospital, London, UK

## Introduction

Clinically non-functioning pituitary tumours (NF-PitNETs) are the most frequently operated pituitary neuroendocrine tumours (PitNETs), and the only ones with no available medical treatment. First-generation somatostatin analogues showed no clinical benefit, and dopamine receptor 2 (D2DR) agonists elicit contradictory results.

D2DR is expressed in a third of hormone negative tumours[1], and some clinical data suggest beneficial effect in patients with tumours remnants: cabergoline (vs no treatment) showed tumour shrinkage 29% vs 10%, stabilization in 66% vs 73%, enlargement in 5% vs 16%, progression-free survival 23 vs 21 months[2]. However, cabergoline responsiveness has not been associated to D2DR presence[2,3]. As some NF-PitNETs might respond[2-4], a biomarker able to predict dopamine agonist responsiveness would be clinically beneficial. Therefore, we tested the effects of cabergoline on primary culture of human NF-PitNETs samples.

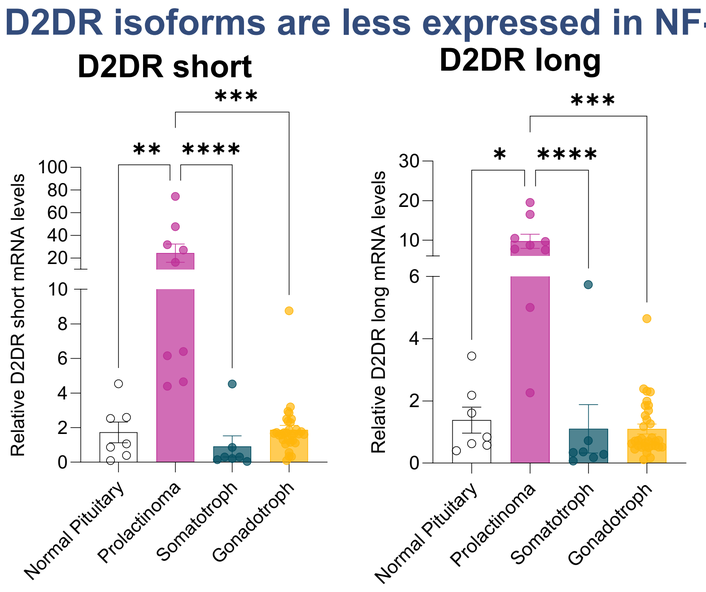



## Materials and Methods

Patients' samples: NF-PitNETs n=33 (gonadotroph tumours), prolactinomas n=10 (clinically partially resistant), somatotroph tumours n=8, normal pituitaries n=7 (autopsy). DRD2 isoforms, Gαi2, and β-arrestin2 expression was tested by qPCR (Taqman probes).

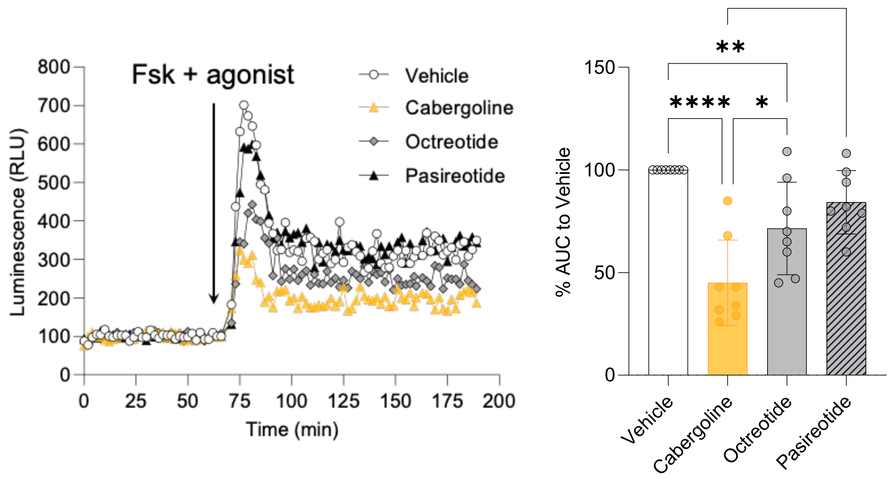
## Results

### D2DR isoforms are less expressed in NF-PitNETs



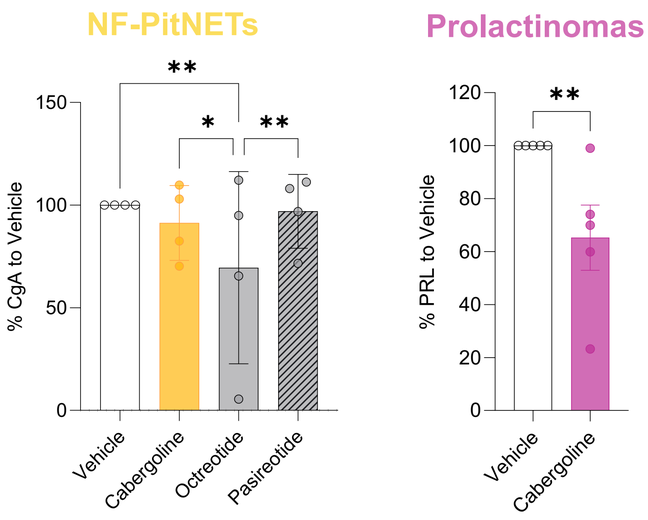

RT-qPCR showing the the expression levels of the two D2DR isoforms, short (left) and long (right) in normal pituitaries (white, n=7), prolactinomas (pink, n=8), somatotroph tumours (blue, n=7), and NF-PitNETs (yellow, n=33). Kruskal-Wallis & Dunn's test.

### Cabergoline and octreotide affect cAMP production in NF-PitNETs upon Forskolin stimulation




PgGlo cAMP assay. Left: a representative curve of the luminescence over time, indicated by the arrow is the compounds and forskolin (Fsk) addition. Right: area under the curve (AUC) expressed as % of vehicle for n=8 NF-PitNETs. 2-way ANOVA & Tukey's test.

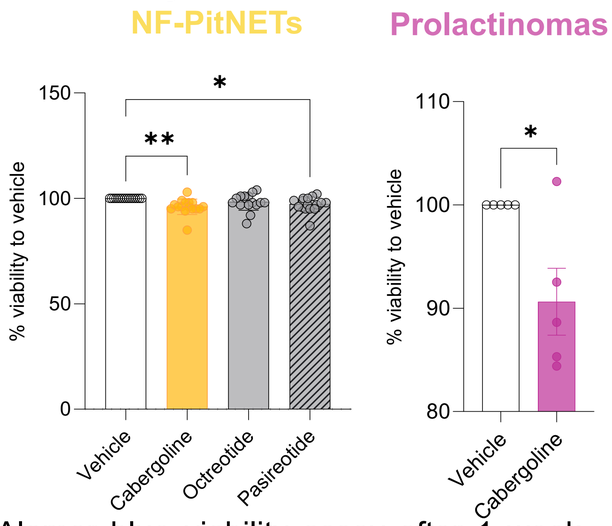
### Cabergoline does not inhibit chromogranin A release In NF-PitNETs




Chromogranin A (CgA) ELISA assay on NF-PitNETs cells treated for one week with cabergoline, octreotide or pasireotide (all 100nM, n=5, 2-way ANOVA & Tukey's test).

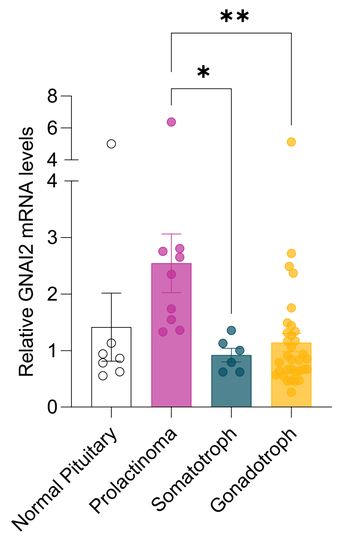
Prolactin (PRL) ELISA assay on prolactinoma cells treated for one week with 100nM cabergoline (n=5, Kolmogorov-Smirnov t-test).

### Cabergoline has only minor effects on viability in NF-PitNETs



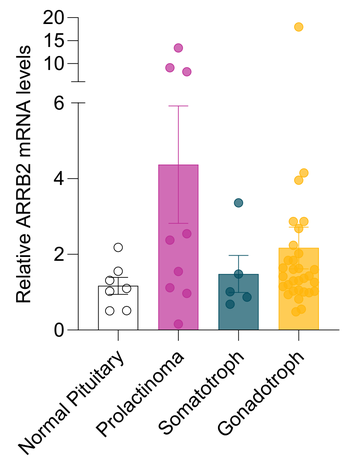

Alamar blue viability assay after 1 week treatment with cabergoline, octreotide, or pasireotide (all 100nM). Left: NF-PitNETs (n=16, 2-way ANOVA & Tukey's test), right: prolactinomas (n=5, Kolmogorov-Smirnov t-test).

### Inhibitory G protein expression is lower in NF-PitNETs compared to prolactinomas




RT-qPCR for Gαi2 (GNAI2), in normal pituitaries (white, n=7), prolactinomas (pink, n=8), somatotroph tumours (blue, n=8) and NF-PitNETs (yellow, n=42; Kruskal-Wallis & Dunn's test).

### NF-PitNETs have similar β-arrestin2 expression compared to resistant prolactinomas




RT-qPCR for β-arrestin2 (ARRB2), in normal pituitaries (white, n=7), prolactinomas (pink, n=8), somatotroph tumours (blue, n=8) and NF-PitNETs (yellow, n=42; Kruskal-Wallis & Dunn's test).

## Conclusions

- **Cabergoline reduces cAMP production in NF-PitNETs cells; however, this does neither correspond to a reduction of chromogranin A release, nor to a strong inhibition of viability, in contrary to prolactinomas.**
- **NF-PitNETs have lower expression of most of the components of the D2DR signalling cascade (long/short D2DR and the inhibitory G-protein Gαi2) compared to prolactinomas. This may explain the lack or partial effect of cabergoline.**
- **NF-PitNETs have similar levels of β-arrestin2 compared to prolactinomas. Since all the prolactinomas tested are partially resistant to cabergoline, this could explain the resistance in both tumour types and therefore could be used as prognostic biomarker.**

## References

1. Wang, Y., Li, J., Tohti, M., *et al*. (2014) The expression profile of Dopamine D2 receptor, MGMT and VEGF in different histological subtypes of pituitary adenomas: a study of 197 cases and indications for the medical therapy. Journal of Experimental and Clinical Cancer Research 33, 56.
2. Batista, R.L., Musolino, N.R.C., Cescato, V.A.S., *et al.* (2019) Cabergoline in the Management of Residual Nonfunctioning Pituitary Adenoma: A Single-Center, Open-Label, 2-Year Randomized Clinical Trial. American Journal of Clinical Oncology 42, 221-227.
3. Greenman, Y., Cooper, O., Yaish, *et al.* (2016) Treatment of clinically nonfunctioning pituitary adenomas with dopamine agonists. European Journal of Endocrinology 175, 63-72.
4. Peverelli, E., Treppiedi, D., Mangili, F., et al. (2021) Drug resistance in pituitary tumours: from cell membrane to intracellular signalling. Nat Rev Endocrinol 17(9):560-571.