Treatment of clinically non-functioning pituitary adenomas with dopamine agonists

Greenman Y¹, Cooper O⁶, Yaish I¹, Robenshtok E⁴,⁵, Sagiv N¹, Jonas KT³,⁵, Yuan X⁷, Gertych A⁷,
Shimon I⁴,⁵, Ram Z²,⁵, Melmed S⁶, Stern N¹,⁵

¹Institute of Endocrinology, Metabolism and Hypertension, ²Department of Neurosurgery, and
³Neuroradiology Unit, Tel Aviv Sourasky Medical Center; ⁴Institute of Endocrinology and Metabolism,
Rabin Medical Center, and ⁵Sackler Faculty of Medicine, Tel Aviv University, Israel. ⁶Pituitary Center,
and ⁷Pathology Department, Cedars Sinai Medical Center, Los Angeles, USA.

Short Title: Dopamine agonists for non-functioning adenomas

Keywords: dopamine agonists, non-functioning pituitary adenoma, medical therapy, pituitary tumor

Word count: 3659

Corresponding author:

Yona Greenman MD

Institute of Endocrinology, Metabolism and Hypertension

Tel Aviv Sourasky Medical Center

6 Weizmann Street, Tel Aviv 64239, Israel

Phone: +972 524262850

Fax: +972 3 6973503

E-mail: yonagr@tlvmc.gov.il
Objective:
Clinically nonfunctioning pituitary adenoma (NFPA), remain the only pituitary tumor subtype for which no effective medical therapy is available or recommended. We evaluated dopamine agonist (DA) therapy for preventing growth of post-surgical pituitary tumor remnants.

Design:
Historical cohort analysis of clinical results at two pituitary referral centers with different standard practices for post-operative NFPA management: DA therapy or conservative follow-up.

Methods:
Seventy nine patients followed for 8.8 ± 6.5 years were treated with DA, initiated upon residual tumor detection on postoperative MRI (preventive treatment (PT) group, N=55), or when tumor growth was subsequently detected during follow-up (remedial treatment (RT) group, N=24). The control group (N=60) received no medication. Tumoral dopamine and estrogen receptor expression assessed by quantitative RT-PCR and immunostaining were correlated with response to treatment.

Results:
Tumor mass decreased, remained stable or enlarged respectively in 38%, 49% and 13% of patients in the PT group, and in 0%, 53% and 47% of control subjects; shrinkage or stabilization was achieved in 58% of enlarging tumors in the RT group, p<0.0001.

15 year progression-free survival rate was 0.805, 0.24 and 0.04 respectively for PT, RT and control groups; p<0.001. 42 % of patients in the control group required additional surgery or radiotherapy, compared to 38% and 13% subjects in the RT and PT groups respectively (p=0.002). Outcome measures were not related to NFPA D2R abundance.

Conclusions:
Dopamine agonist therapy in patients with NFPA is associated with decreased prevalence of residual tumor enlargement after transsphenoidal surgical resection.
INTRODUCTION

Non-functioning pituitary adenomas (NFPAs) are defined by the absence of elevated circulating pituitary hormones, as well as features secondary to tumor-related hormone hypersecretion. They mostly synthesize but rarely secrete gonadotropins or gonadotropin hormone subunits. Hence, NFPA remain clinically silent until significant mass expansion has already occurred. They are typically large at diagnosis, often compressing critical neighboring structures, causing hypopituitarism, visual compromise and cranial nerve palsy (1). Transsphenoidal resection is the preferred treatment, allowing decompression and rapid symptom amelioration in most cases (2). Nevertheless, as these tumors are mostly invasive macroadenomas, a complete resection is challenging. Most patients harbor residual post-operative tumor tissue, which, if left untreated, is associated with progression rates of over 40% over 5-10 years (3-5). Radiation therapy may be effective in preventing residual tumor growth (6), but is associated with a high rate of complications, thus limiting routine use (7, 8). Reoperation for tumor recurrences is often necessary, particularly when there is risk for compromised optic function. Although major surgical complications are infrequent, they are not negligible and include new onset hypopituitarism, cerebrospinal fluid leak, meningitis, cranial nerve injury, and visual compromise, with a mortality rate of 0.3-0.5% (9).

NFPA comprise the most common form of pituitary macroadenomas requiring surgical intervention, yet they remain the only pituitary tumor subtype for which no medications are effective. This contrasts with effective drugs available (10, 11) for treatment of hormone secreting pituitary tumors. Most NFPA express dopamine receptors, predominantly dopamine receptor 2 (D2R) (12, 13). Thus, dopamine agonists (DA) reduce gonadotropin secretion (14) and inhibit thymidine incorporation \textit{in vitro} (15), providing a potential therapeutic target for NFPA. However, dopaminergic binding sites (16) as well as D2RmRNA isoforms (17) are less abundant in NFPA than in prolactinomas, perhaps accounting for the modest rate of tumor shrinkage attained by DAs (18). Small studies, including a report from our
own group (19) suggested that DA may retard NFPA growth (20-24), but to date, this potential therapeutic modality has not been broadly practiced.

We sought to determine the impact of routine DA treatment on progression of post-operative tumor remnants. We hypothesized that such treatment would reduce the need for subsequent interventions such as radiotherapy and repeat surgery with associated risk of added complications. We therefore retrospectively analyzed prospectively collected data derived from two separate NFPA patient cohorts from similar metropolitan population bases: in one center, DA are routinely administered after surgery, whereas in another, standard practice for post-operative NFPA care is conservative follow up. We also assessed abundance of tumoral dopamine and estrogen receptor expression as determinants of treatment response.

SUBJECTS AND METHODS

Patients

The study included NFPA patients from two pituitary referral centers in central Israel distanced < 9 km from each other. Eligible adults (>18 years of age) had residual tumor visible in the first post-operative MRI, with a minimum one year follow up. Exclusion criteria included silent corticotroph and silent somatotroph tumors, and previous history of radiation therapy.

Two patient populations were studied (Figure 1):

1) Treatment group: patients followed at Tel Aviv-Sourasky Medical Center between 1989 and 2013. One hundred and fourteen patients were initially identified and 79 were available for final analysis. Initial follow up of 33 of these patients has been reported (19).
2) Control group: patients followed at Rabin Medical Center between 1995 and 2012. Eighty-seven patients were initially identified and 60 were available for analysis. Characteristics of this cohort have been described (25).

The study was approved by the respective independent institutional ethics committees and complied with the Declaration of Helsinki. Written informed consent was obtained before surgery from patients whose tumor samples were subsequently analyzed. A waiver of written consent was approved for retrospective data extraction from clinical records.

Treatment protocol

Preventive treatment (PT) group: Patients in whom a clear tumor remnant was evident in the first postoperative MRI were offered the option of DA treatment for prevention of subsequent tumor progression. Patients who declined medical therapy were followed conservatively i.e. no active therapy. During 1989 to 2001 bromocriptine was administered, and since 2002 cabergoline was used in all but two patients. Dosages were gradually increased, according to tolerability, with the aim of reaching 10 mg qd of bromocriptine (mean 6.8 ± 2.6 mg, range 2.5-10 mg) and 2 mg weekly of cabergoline (mean 1.5±0.7 mg, range 0.5-3.5 mg). Twenty one out of 55 evaluable patients in the PT group were initially treated with bromocriptine and 14 subsequently switched to cabergoline after a mean period of 4.6 ± 3.2 years (three due to tumor growth while receiving bromocriptine and 11 for patient convenience). The mean time from surgery to medical treatment initiation was 0.8 ± 0.85 years.

Remedial treatment (RT) group: This group comprised patients who likewise had residual masses on post-operative MRI, initially declined medical therapy, but consented once tumor progression was detected on subsequent MRI studies. Medical treatment was as described for the PT group, reaching a mean daily bromocriptine dose of 7.5 ± 2.8 mg (range 2.5-10 mg) and a mean weekly cabergoline dose of 1.4±0.7 mg (range 0.5-3.5 mg). Twelve out of 24 patients in the RT group were initially treated with bromocriptine and 6 subsequently switched to cabergoline after a mean period of 3.34 ± 3.3 years (two
due to tumor growth while receiving bromocriptine and 4 for convenience). The mean time from surgery to medical treatment initiation was 4.2 ± 3.2 years.

**Endpoints and assessments**

The primary endpoint was prevention of tumor progression. Secondary endpoints were tumor shrinkage and the number of clinically required additional interventions (surgery and radiotherapy) during follow-up.

**Imaging protocol:** MRI was performed 3-6 months after surgery and yearly thereafter in all patients. Subjects in treatment groups also underwent MRI 6 months following medical therapy. MRI scans were performed with gadolinium contrast, and evaluated by two experienced neuroradiologists at each institution. Maximal tumor diameters in each plane were used for comparison between MRI scans. Comparisons were performed with the most recent previous images, and also with antecedent available scans. A change in tumor size was considered significant and recorded as such if a difference of at least 2 mm in diameter was observed.

Patients underwent pituitary function and neuro-ophtalmologic assessment including visual fields prior and 4-6 weeks after surgery. Follow up was conducted every three months during the first year and twice yearly thereafter, or at the discretion of the treating physician. At each visit, vital signs and physical condition were assessed, and reevaluation of pituitary function and vision were performed as per standard clinical practice. Pituitary hormone deficiencies were treated with hormone replacement therapy, except for growth hormone deficiency that was not routinely evaluated or treated. Decisions regarding indication for surgery and/or radiotherapy for patients in whom tumor progression occurred during follow up were determined by the treating physician at each institution according to standard clinical practice.

**Immunohistochemistry**
Immunostaining for intact pituitary hormone expression and Ki-67 proliferative index was performed in the respective clinical pathology laboratories. D2R and estrogen receptor (ER) α and β were immunostained, digitized and quantified at the Cedars-Sinai Translational Research Core in 53 tumor samples of DA treated subjects. The percentage of positive cells and staining intensity was recorded for each slide using the immunoreactivity scoring system (IRS) (26) and the (QUICKScore) technique (27) (Supplemental Methods).

Quantitative RT-PCR

Quantification of D2R (long and short isoforms) as well as ER α and β mRNA (Supplemental Table 1 and Supplemental Methods) was performed in 18 tumor samples of DA treated patients (14 from PT and 4 from RT groups), for whom frozen tissue was available for analysis in the institutional tumor bank.

Statistical analysis

The association between tumor characteristics and changes in tumor size by treatment group was examined using the Fisher’s exact test for categorical variables. The rank-sum (Mann-Whitney) test and Analysis of Variance (ANOVA) were used for between group comparisons for numerical variables as appropriate. Results are expressed as mean ± SD. The Cox Proportional-Hazards model was utilized to assess independent associations of different parameters with tumor enlargement. Variables associated with tumor enlargement with a significance level of <0.1, in addition to age and sex, were included in the multivariate Cox Proportional-Hazard models. Schwartz Bayesian Information Criteria were used for selection of the best fit interaction model. Time to detection of tumor enlargement and median recurrence-free survival times were estimated using the Kaplan-Meier method. Tumor progression-free survival was measured from the date of surgery until tumor growth detection in control and PT groups, and from DA treatment initiation until tumor growth detection in the RT group. Data were censored at the date of the last follow-up visit. The log-rank test with Bonferroni adjustment for multiple comparisons was used to
compare progression-free survival curves. Pearson correlation analysis was performed to study correlations between receptor expression levels. P values of less than 0.05 were considered to indicate statistical significance. Data analysis was performed with the SAS 9.3 software (SAS Institute Inc., Cary, NC, USA.).

RESULTS

Patient characteristics

All patients had pituitary macroadenomas before surgery, with a mean (range) maximal diameter of 26.2 mm (10-55), 27.7 mm (13-47) and 28.8 mm (12-90) in the control, remedial (RT) and preventive treatment (PT) groups respectively (Table 1). No significant differences were evident in other tumor features such as presence of invasiveness or hormone immunostaining pattern (Table 1). Baseline characteristics including age, gender, presence of hyperprolactinemia and visual field defects were similar in all groups, but hypopituitarism was more common in the treatment groups (p= 0.037; p=0.042 for RT vs. control group, p=0.053 for PT vs. control group), possibly reflecting different thresholds for hypopituitarism diagnosis between the two medical centers, as well as the longer follow up time in the RT group (p<0.0001; Table 1). Although follow up time in the RT group (13.3 ± 6.3 y) was longer than in the PT (6.7± 5.6 y) and the control groups (6.3 ± 5.2 y), follow up after treatment initiation in the RT group (6.7 ±4.7 years), was similar to that in the other groups (Table 1). Tumor remnants larger than 10 mm were more prevalent in the PT group (88.2%), compared to the RT (75%) and control (63.6%) groups, p=0.016; PT vs. control, p=0.0068 (Table 1).

Primary endpoint: prevention of tumor remnant progression
Tumor control was achieved in 87.3% of the PT group (tumor shrinkage in 38.2% and tumor stabilization in 49.1% of patients) but only in 46.7% of the control group (p<0.0001, Table 2).

In the RT group, in which clear evidence of tumor growth had already been documented in all patients prior to initiation of DA, tumor control was still achieved in 58.4% of them (shrinkage in 29.2% and stabilization in 29.2%), despite the unfavorable pre-selection (Table 2). Escape from therapy was encountered in two patients who initially exhibited tumor shrinkage in the PT group, 30 months and 20 years after treatment initiation respectively.

Patients who experienced tumor growth during follow up, both in the treated and untreated groups, were younger than subjects whose tumors remained stable (54.3 ± 14.1 years, range 27-55, vs. 59.4 ± 13.5, range 27-88, respectively; p = 0.03). In patients receiving preventive treatment, pre-operative maximal tumor diameter was significantly larger (37± 35 mm) in the progressing tumors than in the controlled tumors (27.5± 9 mm), p=0.047). Presence of hyperprolactinemia, hypopituitarism, remnant size and immunostaining characteristics were not related to outcome.

Tumor-progression free median survival was 6 years (95% CI5-7) for the control group, 8.5 years (95% CI 3.1-Not Computable) for the RT group, and could not be estimated in the PT group since after 24 follow-up years the rate of tumor progression was less than 50% (p <0.0001; Figure 2). Actuarial tumor progression free survival at 5 years was 0.69, 0.88 and 0.6 in the control, PT and RT groups respectively (p=0.052; p=0.04 (PT vs. RT)). Ten year progression free survival rate was 0·81, 0.48 and 0.12 for PT, RT and control groups respectively (p=0.0002; p=0.0001 (PT vs. control), p=0.06 (RT vs. control) and p=0.02 (PT vs. RT)). Fifteen year progression-free survival rate was 0·81, 0.24 and 0.04 for PT, RT and control groups respectively (p<0.0001; p<0.0001 (PT vs. control), p=0.04 (RT vs. control) and p=0.0053 (PT vs. RT)).

The Cox Proportional-Hazards model was used to examine treatment effect on occurrence of tumor enlargement. Parameters entered into the model were treatment group, age, sex, as well as tumor size and
invasiveness prior to surgery and post-operative remnant size. Hazards ratio for growth in treatment vs. control groups was 0.3 (95% CI 0.16-0.56; p = 0.0002), after adjustment for gender (HR for male vs. female gender 2.2; 95% CI 1.18-4.09; p=0.012) and age (HR for each increasing year 0.96; 95% CI 0.94-0.98, p=0.001). Within the treatment arm, the hazards ratio for tumor progression in PT vs. RT groups was 0.273 (95% CI 0.1-0.74, p=0.011) after adjustment for preoperative maximal tumor diameter (in mm) (HR 1.049, 95% CI 1.008-1.09; p=0.02), age (HR 0.96, 95% CI 0.93-0.99, p=0.02) and sex (HR 2.17 for male gender, 95% CI 0.586-8).

Secondary endpoints

Clinical events

Twenty five patients (41.7 %) in the control group, compared with 9 (37.5%) and 7 (12.7%) in the RT and PT groups respectively required additional surgery and/or radiotherapy (p=0.002), with a total event rate of 46.7%, 50% and 16.4% respectively (p= 0.0008, table 3). One of three patients in the PT group and one of two patients in the RT group who experienced tumor progression while receiving bromocriptine treatment had stabilized tumor growth after switching to treatment with cabergoline. One patient in the PT group developed nausea and dizziness shortly after initiating DA therapy, leading him to drop out of the study (Figure 1). After reaching the maximal tolerated DA dose, no adverse events attributable to DA therapy were identified during long term follow up.

Correlation between clinical outcome and tumor D2R expression

Tissue was available for immunostaining in 53 of 79 patients in the treatment groups (36 and 17 in the PT and RT groups respectively). D2R staining was positive in the majority of cells (>75 %) in 53% of tumor samples. On the other hand, only 15% of samples exhibited scarce D2R staining (< 25% of cells). The IRS score, that integrates the percentage of positively stained cells with the intensity of staining, was high (upper quartile) in 77 % of tumor samples (Supplemental table 2). Primary or secondary outcome measures were not related to D2R tumor abundance (Figure 3a and 3b, Supplemental Tables 2 and 3), or
to D2R mRNA isoform expression levels (Figure 3c). Interestingly, D2R mRNA isoform expression was higher in DA resistant prolactinomas (2.1 ± 0.2 and 4.2 ± 0.5 1/2Δ Ct for long and short isoforms respectively) than in DA responsive NFPA (0.7 ± 0.3 and 1.6 ± 0.7 1/2Δ Ct for long and short isoforms respectively), p<0.001, Figure 3c.

ERα staining was undetectable in 47% of tumor samples. Only three samples stained positively for ERα in 25-75% of tumor cells. In contrast, ERβ was expressed in 90% of samples, half of which expressed the receptor in all cells (100%). ER α and ER β staining scores (Supplemental tables2 and 3), and mRNA levels (Supplemental Fig. 1) as well as Ki-67 immunopositivity (Supplemental Table 2) in tumor remnants did not differ according to their response to DA treatment.

There was a significant and strong positive correlation between mRNA expression levels of the long and short D2R isoforms (r= 0.941; 95% CI 0.847-0.978; p<0.0001) and between mRNA expression levels of ER α and ER β (r=0.57; 95% CI 0.15-0.82; p=0.012). This uniformity in D2R isoform and ER expression in NFPA samples supports the finding that the possible preponderant expression of one isoform over the other may not be an important determinant of tumoral response to DA therapy, as previously suggested.

DISCUSSION

Faced with a tumor remnant after incomplete surgical resection of NFPA, the current consensus guidance for practice is expectant follow up and the use of radiotherapy or repeat surgery if required (1, 29, 30). This "wait and see" strategy reflects the often unacceptable rate of long term complications of radiation therapy as well as the usual indolent course of the disease. However, tumor progression in untreated patients occurs commonly and is often unpredictable (31) with variably delayed recurrence and most importantly, not consequence-free. Secondary interventions such as repeated surgery and radiotherapy are then implemented, not always under clinically optimal circumstances and carry the risk of concurrent associated morbidity.
In this study we demonstrated the efficacy of DA therapy in this clinical setting, and as compared to untreated patients, preventive treatment with DA reduced the occurrence of tumor progression by 76%.

Furthermore, even when treatment was administered to patients in whom there was already evidence for active tumor growth, it still lowered the incidence of tumor progression, and induced tumor shrinkage or stabilization in over 58% of patients subjected to delayed, rather than preventive DA therapy. Response to treatment was prolonged, with a very low rate of escape (1.6%) during a long term follow up.

Importantly, the requirement for additional surgery and radiation during follow up decreased from 46.7% to 16.4% with preventive treatment, indicating that the observed, DA treatment-related decline in tumor progression translated to clinically significant benefits. This reduction in the need for additional interventions is of particular importance in view of the recently reported association between multiple surgeries or surgery combined with RT, with elevated standardized mortality ratios (2.67) reported in patients with NFPA (32). Our results lead us to propose that currently practiced expectant follow up in subjects with NFPA with residual post-operative remnant, even in the absence of evident mass effects on vital structures, is less beneficial than preventive treatment with DA.

The use of DA for the treatment of NFPA has been explored for over 30 years, but results are derived from case reports and small series, including heterogeneous patient populations, with follow up times usually shorter than one year, using different dosages of medication, mostly bromocriptine. Colao et al (18) analyzed 24 studies published between 1981 and 2005, encompassing 199 patients and reports stabilization of over 90% of NFPA masses in patients treated with DA. Five studies (20-24) encompassing 54 patients used cabergoline (mean dose 1-3 mg/week) for treatment of NFPA (primary treatment or post-surgical remnants) with mean follow up times between 6 and 12 months. Pooled results indicate that tumor stabilization occurred in 85% of patients (supplemental table 4). Albeit encouraging, the lack of untreated control groups in most published studies, the possibility of selection bias, and the small number of patients in each series does not allow for firm conclusions as to treatment effectiveness or tumor characteristics that may influence response to treatment.
Although our study is also limited in its design and a double blind, randomized placebo-controlled study (RCT) would have been preferable, under "real world" circumstances, the design of our study should be weighed against the practical alternatives. First, the probability that such a study with generic DA will be performed is low due to the lack of commercial incentive. Second, our report is based on a long-term study, overall encompassing 20 years. Given the slow growing nature of these tumors and the lack of serum markers to reflect treatment effectiveness, even if a publicly funded RCT is initiated, it would require a protracted follow up time. We minimized patient selection bias by using a design in which the studied patient populations were derived from two different medical centers within a similar geographical, ethnic and cultural area, which to the best of our judgment, differ only with respect to the different management protocols which are the subject of the present report. Of note, the use of radiation therapy in the post-operative management of NFPA was also never studied in a RCT, and a similar design as in our study was utilized to investigate its effectiveness in this clinical context (6).

In contrast to the low D2R expression characteristic of prolactinomas resistant to DA therapy (33), we found no correlation between clinical response to DA treatment and D2R expression, as evaluated at the protein level by immunocytochemistry and, in a smaller number of cases, at the mRNA level. These results are in accordance with a previous report in which dopamine D2R imaging using 123I-epidepride had limited clinical usefulness for predicting the efficacy of DA treatment in NFPA (22). Discordant studies reported association of dopamine resistance in prolactinomas with low expression levels of the short (34) or long (35) isoforms of D2R. In NFPA, an association between the (qualitative) expression of the D2R short isoform with response to DA in vitro (36), and in vivo (21) has been suggested. We did not find an association between either the isoform type or expression levels with clinical response to medical treatment in NFPA. Possibly a larger number of clinical and tumor correlates could shed more light on this question. At this point, we cannot explain the lack of correlation between dopamine receptor expression levels and response to DA treatment in NFPA. Additional factors potentially associated with clinical response to DA treatment in prolactinomas such as D2R polymorphisms, Nerve Growth Factor...
receptor expression, alterations in cellular signaling factors down-stream of D2R such as decreased levels of $G_{\alpha i}$ inhibitory G protein subunit (33) have yet to be analyzed.

Another important limitation of this study is the inherent variability in imaging interpretation at the 2 centers. Nevertheless, such variability would not account for the large differences in the requirement for repeated surgery and radiation observed between the groups, which can derive only from clear-cut and clinically relevant tumor enlargement. Finally, the possible safety concern regarding high dose cabergoline-induced valvular heart disease (37) has not been reported with lower doses such as those used in our study, and in pituitary disorders in general (38) and therefore appears not to weaken the significance of our report.

In conclusion, dopamine agonist therapy was associated with decreased incidence of post-operative residual tumor enlargement in patients with NFPA. We propose that this treatment modality be considered for management of these patients.

**Declaration of interest:** Authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

**Funding**

This work was supported by an Israel Endocrine Society Young Investigator Award; the Doris Factor Endowment Trust; Novartis Pharma (Investigator Initiated Trial Award); National Institute of Diabetes and Digestive and Kidney Diseases (Grant K23DK085148); National Center for Advancing Translational Sciences, Grant UL1TR000124 (The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH).
REFERENCES


Metabolism Clinics of North America* 2015 44 51–70.

subtypes in normal human pituitaries, nonfunctioning pituitary adenomas and somatotropinomas,
and the association between dopamine and somatostatin receptors with clinical response to
octreotide-LAR in acromegaly. *Journal of Clinical Endocrinology and Metabolism* 2009 94
1931-1937.

Dopamine 2 receptor expression in various pathological types of clinically non-functioning

Bromocriptine increasingly suppresses the in vitro gonadotropin and alpha-subunit release from
pituitary adenomas during long term culture. *Journal of Clinical Endocrinology and Metabolism*
1990 71 718-724.

GK, Theodoropoulou M, Culler MD, et al. Efficacy of a dopamine-somatostatin chimeric
molecule, BIM-23A760, in the control of cell growth from primary cultures of human non-
functioning pituitary adenomas: a multi-center study. *Endocrine Related Cancer* 2008 15 583-
596.

16. Bevan JS & Burke CW. Non-functioning pituitary adenomas do not regress during bromocriptine
therapy but possess membrane-bound dopamine receptors which bind bromocriptine. *Clinical


24. Vieira Neto L, Wildemberg LE, Moraes AB, Colli LM, Kasuki L, Marques NV, Gasparetto EL, de Castro M, Takiya CM & Gadelha MR. Dopamine receptor subtype 2 expression profile in


FIGURE LEGENDS

Figure 1 - Assessment for eligibility and enrollment in the treatment (1a) and control (1b) groups.

Figure 2 - Actuarial tumor progression free survival in patients with post-operative tumor remnants according to treatment group: Preventive treatment group (PT) - treatment was initiated upon residual tumor detection on postoperative MRI; remedial treatment group (RT) - treatment was initiated upon tumor growth detection during follow-up; control group - untreated. At five years: log rank test p=0.052; PT vs. RT p=0.045; n= NS for other comparisons. At 10 years: log rank test p=0.0002; control vs. PT p=0.0001; control vs. RT p=0.06; PT vs. RT p=0.019. At 15 years: log rank test p<0.0001; control vs. PT p<0.0001; control vs. RT p=0.039; PT vs. RT p=0.0053.

Figure 3- A- Strong (IRS-12) and B- weak (IRS-3) immunostaining for D2R. Both tumors remained stable under DA therapy. Magnifications 4X and 10X. IRS= immunoreactivity score. C: D2R isoform mRNA expression in normal pituitary, DA resistant prolactinoma (n=3) and NFPA tumors according to response to treatment: tumor shrinkage (n=6), stable tumor (n=8) and tumor growth (n=3). p<0.001 for the comparison between prolactinoma and NFPA groups for both isoforms.
Table 1- Baseline Demographic and Clinical Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>Control (n=60)</th>
<th>Remedial Treatment (n=24)</th>
<th>Preventive Treatment (N=55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.3 (14.5)</td>
<td>56.6 (13.7)</td>
<td>58.3 (13.4)</td>
</tr>
<tr>
<td>Sex (Female)</td>
<td>21 (35%)</td>
<td>7 (29.2%)</td>
<td>26 (47.3%)</td>
</tr>
<tr>
<td>Follow up (F/U) time (years)</td>
<td>6.3 (5.2)</td>
<td>13.3 (6.3)</td>
<td>6.7 (5.6)</td>
</tr>
<tr>
<td>F/U time after DA initiation</td>
<td>6.7 (4.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal preoperative tumor</td>
<td>26.2 (10.9)</td>
<td>27.7 (9.5)</td>
<td>28.8 (12.3)</td>
</tr>
<tr>
<td>diameter (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasiveness</td>
<td>35 (58.3%)</td>
<td>13 (54.2%)</td>
<td>32 (58.2%)</td>
</tr>
<tr>
<td>Visual field defect</td>
<td>35 (58.3%)</td>
<td>12 (54.5%)</td>
<td>27 (50.9%)</td>
</tr>
<tr>
<td>N=60</td>
<td>10 (45.5%)</td>
<td>N=21</td>
<td>N=53</td>
</tr>
<tr>
<td>Hyperprolactinemia</td>
<td>14(31.8%)</td>
<td>7 (33.3%)</td>
<td>15 (34.9%)</td>
</tr>
<tr>
<td>N=44</td>
<td>N=21</td>
<td>N=43</td>
<td></td>
</tr>
<tr>
<td>Hypopituitarism</td>
<td>22 (40%)</td>
<td>15 (68.2%)</td>
<td>31 (52.7%)</td>
</tr>
<tr>
<td>N=55</td>
<td>N=22</td>
<td>N=52</td>
<td></td>
</tr>
<tr>
<td><strong>Immunostaining</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gonadotropinoma</td>
<td>15 (31.9%)</td>
<td>12 (54.5%)</td>
<td>16 (31.4%)</td>
</tr>
<tr>
<td>Null cell adenoma</td>
<td>27 (57.4%)</td>
<td>10 (45.5%)</td>
<td>27 (52.9%)</td>
</tr>
<tr>
<td>Other/plurihormonal</td>
<td>5 (10.6%)</td>
<td>0</td>
<td>8 (15.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>N=47</td>
<td>N=22</td>
<td>N=51</td>
</tr>
<tr>
<td><strong>Remnant size</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 mm</td>
<td>16 (36.4%)</td>
<td>6 (25%)</td>
<td>6 (11.8%)</td>
</tr>
<tr>
<td>&gt;10 mm</td>
<td>28 (63.6%)</td>
<td>18 (75%)</td>
<td>45 (88.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>N=44</td>
<td>N=24</td>
<td>N=51</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
</tbody>
</table>

*a Data are means (SD) or numbers (%)*

*b p<0.0001*

*c Defined as invasion of cavernous sinus detected in MRI and quantified over grade 2 in the Knosp classification. (28)*

*d Presence of dysfunction in one or more pituitary axes; p=0.037; p=0.042 for the comparison between RT and control groups, p=0.053 for the comparison between PT and control groups*

*e p=0.016*
Table 2- Effect of treatment on residual tumor progression

<table>
<thead>
<tr>
<th></th>
<th>Control (n=60)</th>
<th>Remedial Treatment (n=24)</th>
<th>Preventive Treatment (N=55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor growth</td>
<td>32 (53.3%)</td>
<td>10 (41.6%)</td>
<td>7 (12.7%)</td>
</tr>
<tr>
<td>Tumor stabilization</td>
<td>28 (46.7%)</td>
<td>7 (29.2%)</td>
<td>27 (49.1%)</td>
</tr>
<tr>
<td>Tumor shrinkage</td>
<td>0</td>
<td>7 (29.2%)</td>
<td>21 (38.2%)</td>
</tr>
<tr>
<td>Overall tumor control</td>
<td>28 (46.7%)</td>
<td>14 (58.4%)</td>
<td>48 (87.3%)</td>
</tr>
<tr>
<td>(shrinkage + stabilization)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P<0.0001
Table 3- Clinical outcomes

<table>
<thead>
<tr>
<th></th>
<th>Control (n=60)</th>
<th>Remedial Treatment (n=24)</th>
<th>Preventive Treatment (N=55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation therapy</td>
<td>12 (20%)</td>
<td>4 (16.7%)</td>
<td>3 (5.5%)</td>
</tr>
<tr>
<td>Additional surgery</td>
<td>16 (26.7%)</td>
<td>8 (33.3%)</td>
<td>6 (10.9%)</td>
</tr>
<tr>
<td>Total events&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>28 (46.7%)</td>
<td>12 (50%)</td>
<td>9 (16.4%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Three, three and two patients in the control, RT and PT groups had both additional surgery and radiation therapy

<sup>b</sup>p=0.0008
Figure 1a- Treatment Group

Assessed for eligibility (n=114)

Excluded (n= 31)
- Not meeting inclusion criteria (n=28) (14 without enough follow up data, 7 after radiation therapy and 7 without tumor remnant)
- Declined to participate (n=3)

Initial Cohort (n= 83)

Preventive treatment (PT) group (n=56)
- Discontinued intervention (side effects to treatment) (n=1)
  - Analysed (n= 55)

Conservative follow up (n=27)
- Stable remnants (n=3)
  - Remedial treatment (RT) group (n=24)
    - Analysed (n= 24)
Figure 1 b- Control group

Assessed for eligibility (n= 87)

Excluded (n=27)
- Not meeting inclusion criteria (n=27)
  (23 without enough follow up data,
   6 after radiation therapy and 8
   without tumor remnant)

Analysed (n= 60)
Figure 2

[Graph showing survival rates over time for different treatments]