

**MEN1 Gene Replacement Therapy Reduces Proliferation Rates in a Mouse Model of Pituitary Adenomas**

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**Abstract**

Multiple endocrine neoplasia type 1 (MEN1) is characterized by the combined occurrence of pituitary, pancreatic, and parathyroid tumors showing loss of heterozygosity in the putative tumor suppressor gene MEN1. This gene encodes the protein menin, the overexpression of which inhibits cell proliferation in vitro. In this study, we conducted a preclinical evaluation of MEN1 gene therapy in pituitary tumors of Men1⁺⁻ mice, using a recombinant nonreplicating adenoviral serotype 5 vector that contained the murine Men1 cDNA under control of a cytomegalovirus promoter (Men1rAd5). Pituitary tumors in 55 Men1⁺⁻ female mice received a transaucular intratumoral injection of Men1rAd5 or control treatments, followed by 5-bromo-2-deoxyuridine (BrdUrd) in drinking water for four weeks before magnetic resonance imaging (MRI) and immunohistochemical analysis. Immediate procedure-related and 4-week mortalities were similar in all groups, indicating that the adenoviral gene therapy was not associated with a higher mortality. Menin expression was higher in the Men1rAd5-treated mice when compared with other groups. Daily proliferation rates assessed by BrdUrd incorporation were reduced significantly in Men1rAd5-injected tumors relative to control-treated tumors. In contrast, apoptotic rates, immune T-cell response, and tumor volumes remained similar in all groups. Our findings establish that MEN1 gene replacement therapy can generate menin expression in pituitary tumors, and significantly reduce tumor cell proliferation. *Cancer Res*; 72(19); 5060–8. ©2012 AACR.

**Introduction**

Multiple endocrine neoplasia type 1 (MEN1), which is an autosomal dominant disorder, is characterized by the combined occurrence of tumors of the parathyroid, pancreatic islets (e.g., gastrinomas and insulinomas) and anterior pituitary (e.g., prolactinomas and somatotrophinomas; ref. 1). In addition, some patients with MEN1 develop adenocortical tumors, foregut carcinoids, lipomas, meningiomas, facial angiofibromas, and collagenomas (1). Treatment of pancreatic islet cell tumors, anterior pituitary adenomas, and foregut carcinoids, which comprise the neuroendocrine tumors (NET) in MEN1 patients, is more difficult than for those same tumors in non-MEN1 patients, for several reasons (1–3). First, the MEN1 tumors, with the exception of anterior pituitary adenomas, are multiple. For example, in MEN1 patients, unlike non-MEN1 patients, multiple submucosal duodenal and pancreatic gastrinomas develop, thereby reducing the success rates for surgery, such that only approximately 15% of MEN1 patients, compared with approximately 45% of non-MEN1 patients, are free of disease immediately after surgery, and at 5 years this number has decreased to approximately 5% in MEN1 patients, compared with approximately 40% in non-MEN1 patients (1, 4, 5). Second, NET metastatic disease, which may be occult, is more prevalent in MEN1 patients than in patients with sporadic endocrine tumors; for example metastases may be present at the time of presentation in up to 50% of patients with MEN1-associated insulinomas, whereas the lifetime risk of metastatic insulinoma in non-MEN1 patients is less than 10% (1, 5). Third, the majority (~80%) of NETs have low proliferation rates, with a Ki-67 index of less than 2% (6, 7), and as such are not responsive to chemotherapy or radiotherapy. Fourth, NETs in MEN1 are larger, more aggressive, and resistant to treatment (2, 3).

For example, approximately 85% of anterior pituitary adenomas in MEN1 patients, as opposed to 64% in non-MEN1 patients (8), are macroadenomas: approximately 30% of anterior pituitary adenomas in MEN1 patients have invaded surrounding tissue (Hardy classification grades III and IV), compared with 10% in non-MEN1 patients (9); and more than 45% of anterior pituitary adenomas in MEN1 patients had persistent hormonal oversecretion following appropriate medical, surgical, and radiotherapy treatments compared with between 10% and 40% in non-MEN1 patients.
One possible new treatment could be Men1 gene replacement therapy as the majority (>90%) of MEN1 NETs have LOH for the Men1 allele located on chromosome 11q13, consistent with a tumor suppressor role for the encoded 110 amino acid protein, menin (13, 14), which is predominantly a nuclear protein that interacts with proteins that are involved in transcription regulation, genome stability, and cell division (15). For example, menin interacts with JunD to inhibit its transcriptional activity and thereby cell proliferation; and menin interaction with the mixed lineage leukemia protein 1 (MLL1), which is a histone H3 lysine 4 methyl transferase, that functions as an oncogenic cofactor to upregulate gene transcription, promotes MLL1-fusion-protein-induced leukemogenesis (16). MEN1 tumors have been shown to have germline and somatic mutations, consistent with the Knudson two-hit hypothesis, and to lack menin expression (15). Furthermore, in vitro studies in which menin was expressed by the use of recombinant plasmid, adenoviral, or retroviral vectors, in either menin-null mouse embryonic fibroblasts (MEF), RAS-transformed NIH3T3 MEFs, or rat insulinoma cell lines, have shown that menin overexpression can result in decreased cell proliferation and increased apoptosis (17–21). One study, in which RAS-transformed NIH3T3 MEFs that overexpressed menin were injected into athymic nude mice, showed that menin reduced tumor growth (17). We, therefore, explored the feasibility of in vivo Men1 gene replacement therapy in a mouse model for MEN1, using a recombinant replication-deficient adenovirus serotype 5 (rAd5). We chose rAd5 because of its ability to infect dividing and nondividing cells and its high efficiency of transduction and transient gene expression (22, 23); these qualities are well suited for cancer gene therapy where the goal is to achieve short-term expression that destroys abnormal tissue, that consists of dividing and non-dividing cells, which is particularly important for endocrine tumors that are slow growing and have a low rate of cell division (24). Moreover, adenoviral vectors remain epichromosomal and therefore do not cause insertional mutagenesis; can accommodate large quantities of foreign DNA through deletions of the viral genome; replicate efficiently in vitro and can be produced in high titres; are the most commonly used vectors in cancer gene therapy; continue to show increasing promise as gene therapy delivery vehicles; and have been approved for clinical use in phase I and II trials of various cancers including malignant glioma, and those of lung, prostate, and the head and neck (23, 25, 26). These phase I and II trials have established the safety of adenoviral gene vectors in cancer gene therapy; continue to show increasing promise as gene therapy delivery vehicles; and have been approved for clinical use in phase I and II trials of various cancers including malignant glioma, and those of lung, prostate, and the head and neck (23, 25, 26). 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Figure 1. *Men1* recombinant adenoviral vector. A, genomic organization of the *Men1* recombinant vector generated by using the replication-deficient adenovirus serotype 5 (*Men1*.rAd5). The adenovirus has the E1 and E3 regions deleted, thereby making it replication deficient (Ad5ΔE1/ΔE3). The expression cassette consisting of 1833bp *Men1* cDNA, CMV promoter, and polyA* sequence was inserted into the E1 region of the adenoviral genome. The locations of the left and right inverted terminal repeats (LTR and RTR, respectively) together with the PacI restriction endonuclease sites are shown. A recombinant adenoviral vector expressing GFP.rAd5 (not shown) was used as a control. B, detection of menin and GFP expression by Western blot analysis of lysates from *Men1*−/− MEFS following 48 hours of infection with either the *Men1*.rAd5 or GFP.rAdS vectors or treatment with control PBS. Menin (67KDa) and GFP (27KDa) protein expression were detected only in lysates from *Men1*.rAd5- or GFP.rAdS-treated MEFS, respectively. Detection of α-tubulin (55KDa) expression was used as a positive control. C, per cultured cell, respectively, and observed by fluorescence microscopy using an Eclipse E400 microscope (Nikon) as described (37).

**Analysis of DNA and proteins**

*Men1* genotypes were determined using DNA and PCR primers (29, 38), and the vector construct was sequenced using methods previously described (39). Western blot analysis to assess GFP and menin expression was conducted using rabbit antibodies [anti-memin (AbCam), anti-GFP (Santa Cruz Biotechnology) and anti-α-tubulin (AbCam)], and a secondary antibody [horseradish peroxidase-conjugated goat anti-rabbit (Bio-Rad Laboratories)], as previously described (29).

**Delivery of adenoviral vectors to pituitary tumors**

Anesthetized female *Men1*+/− mice with pituitary tumors, identified by cranial MRI (see below), were randomized to receive a 20 μL transauricular, intratumoral injection of *Men1*.rAdS (5 × 10¹⁰ viral particles), GFP.rAdS (5 × 10¹⁰ viral particles), or control PBS (Fig. 2). Accurate targeting of tumors was confirmed, in a subset of treated female *Men1*−/− mice, by MRI immediately after the transauricular injection (Fig. 3A–C), which was delivered using a Hamilton syringe and 30-gauge needle (40). To eliminate any bias in the analysis and 10⁴ (not shown) viral particles per cultured cell. GFP expression was assessed using fluorescent microscopy using an Eclipse E400 microscope (Nikon) as described (37).

Additional control arms included an uninjected group of *Men1*−/− mice who had no tumors on MRI or subsequent necropsy and were not injected (control for age-related mortality). B, mortality at 4 weeks postinjection was similar in the treatment and control groups. The weights of the *Men1*−/− female mice in the different groups were not significantly different (data not shown) and none of the *Men1*−/− mice had dysmorphic features.
interpretation of the results, the investigators were blinded to the contents of the vials containing the solutions for injections. A control noninjected group of female Men1+/−/C0 mice was included in the study (Fig. 2). Four weeks following administration of treatment, the mice had another MRI scan, were sacrificed, a necropsy undertaken, and tumors and tissues harvested.

**MRI**

Mice were anesthetized with isoflurane and MRI of the cranium conducted using a nonionic MRI contrast medium, gadodiamide (Omniscan, Amersham Health AS), which was injected intraperitoneally at 0.1 mmol/kg. An 11.7 Tesla (500 MHz) MR system was used, image reconstruction was conducted using purpose-written software in Matlab (Mathworks), and image data exported into tagged image file format (TIFF) and loaded into Scion Image (Scion Corporation). The MRI scans were coded so that investigators undertaking the analysis did not know whether the images were pre- or posttreatment. Pituitary tumor volumes were quantified as the sum of the area of all 1 mm sections containing the tumor.

**Histology, immunohistochemistry, and analysis**

Histology and immunostaining of tumors and tissues was carried out using previously described methods (29) and appropriate primary and secondary antibodies. Sections were...
counterstained with either hematoxylin or 4,6-diamino-2-phenylindole (DAPI; ProLong Gold Antifade reagent with DAPI; Molecular Probes). Apoptotic cells were labeled using ApopTag Fluorescein In Situ Apoptosis Detection Kit (Chemicon) according to the manufacturer’s instructions. Sections were viewed by light or fluorescent microscopy using an Eclipse E400 microscope (Nikon), and images captured using a DXM1200C digital camera and NIS-Elements BR 2.30 software (both Nikon). Images were acquired at ×20 magnification. Nuclear menin staining was quantified using a 3-point grading system, with no staining = 0; staining in fewer than half the cells = 1; and staining in more than half the cells = 2. BrdUrd-labeled pituitary cell proliferation rates were ascertained from an average of 6 × 20-fields, from each animal.

Statistics

All results were reported as the mean ± SEM for equivalent groups. The mean values of tumor volume variation over the 4-week study and 95% confidence intervals (CI) were calculated for each experimental group. Results were compared with independent t tests (unpaired and 2-tailed) or χ² tests.

Results

Recombinant adenoviral vector construction, infectivity, and expression of menin

DNA sequence analysis (data not shown) confirmed the presence of the murine Men1 1833bp cDNA in the recombinant Men1.rAd5 vector (Fig. 1A). Expression of the 67kDa menin by the Men1.rAd5 was showed by Western blot analysis in Men1⁻/⁻ MEFs (Fig 1B) and in HEK293 cells (data not shown). Furthermore, expression of GFP was shown in Men1⁻/⁻ MEFs that were infected with GFP.rAd5 (Fig. 1B). The ability of the recombinant adenoviral vector to infect Men1⁻/⁻ pituitary tumor cells was assessed by an infectivity test in which increasing amounts of GFP.rAd5 viral particles were used (Fig. 1C). This revealed that the number of pituitary tumor cells, which were infected with GFP.rAd5 increased commensurately with the increased dose of viral particles. Thus, these in vitro results establish that Men1.rAd5 expressed menin and that rAd5 vectors can infect Men1⁻/⁻ MEFs and primary Men1⁻/⁻ pituitary tumor cells.

In vivo effects of Men1.rAd5 vector

We embarked on in vivo studies aimed at investigating the potential antiproliferative effects of Men1 gene replacement therapy, using Men1.rAd5, as our in vitro studies showed menin expression by Men1.rAd5 and the ability of rAd5 vectors to infect Men1⁻/⁻ pituitary tumor cells (Fig. 1). To assess such an effectiveness of the Men1.rAd5 vector, we designed a randomized controlled trial (Fig. 2) in which investigators were blinded to the treatment received by the Men1⁻/⁻ female mice. The presence of pituitary tumors was assessed by MRI using gadodiamide enhancement (Fig. 3A). MRI was conducted in a total of 150 Men1⁻/⁻ female mice, aged 18.5 ± 0.2 months, and anterior pituitary adenomas, ranging in diameter from more than 2 mm to less than 5 mm, were identified in approximately 37% of mice (n = 55) consistent with the previously observed frequency of pituitary tumors at this age in Men1⁻/⁻ mice (29). Twenty-one of the 95 Men1⁻/⁻ mice in which pituitary adenomas were not identified by MRI, had pituitary adenomas identified at necropsy; however these tumors were less than 1 mm in diameter. Overall, these findings indicate that the use of MRI for the detection of anterior pituitary adenomas in mice has a sensitivity of 72% and a specificity of 100%. Histologic examination of the pituitary adenomas revealed that the majority immunostained for prolactin and the remainder immunostained for growth hormone, which is consistent with previous findings (29) and a pars distalis origin for these tumors (29).

The 55 Men1⁻/⁻ mice with pituitary adenomas were randomized to one of the following 4 treatment groups that received a transauricular intratumoral injection of either Men1.rAd5 (n = 15), GFP.rAd5 (n = 14), or PBS (n = 14), or no injection (n = 12; Fig 2A). Accurate targeting of injections to the tumors was confirmed by MRI immediately postinjection (Fig. 3B) in a subset of mice and MRI 24 hours later revealed absorption of the injected solution (Fig. 3C). In addition, 6 of 74 Men1⁻/⁻ mice that did not have MRI-identifiable anterior pituitary adenomas and did not receive any treatment, were retained as age-matched controls (Fig. 2). Five of the 43 Men1⁻/⁻ anesthetized mice (12%) that received the transauricular intratumoral injection died within 10 minutes. Three of these mice had received Men1.rAd5, one had received GFP, rAd5, and one had received PBS. However, this immediate postinjection mortality was not significantly different (P > 0.14, χ² test) in the 3 groups that received an injection. This finding indicates that the immediate mortality was likely because of the trauma of the procedure and anesthesia, rather than a consequence of the rAd5 vectors. A further 8 Men1⁻/⁻ mice died over the next 4 weeks postinjection, distributed among the 3 treatment groups, giving an overall mortality of 21% over the study period. However, the mortality was not significantly different (P > 0.62, χ² test) in any of the treated or nontreated groups: indeed the highest mortality of 25% (n = 3 out of 12 mice) was observed both in the Men1.rAd5 treated group and the age-matched noninjected and anesthetized control group (Fig. 2B). This mortality figure is consistent with that which we previously reported in Men1⁻/⁻ mice of this age (29). These results indicate that mortality in these 18- to 20-month-old Men1⁻/⁻ mice was likely due to old age, rather than a consequence of Men1 gene replacement therapy with the Men1.rAd5 vector.

Successful treatment of tumors may result in a reduction of tumor volume and/or decreased tumor cell proliferation or increased apoptosis, and we assessed for these effects by measuring tumor volume by MRI, BrdUrd incorporation into the DNA of dividing cells, and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay by immunohistochemistry (IHC), respectively (Figs. 3 and 4). MRI assessment of pre- and posttreatment pituitary tumor volumes in the 3 groups receiving transauricular intratumoral injections did not reveal any significant differences (Fig. 3D). The absence of a significant reduction in pituitary tumor volume in the Men1.rAd5 group may be because of several reasons. First, the Men1 gene may not be stably expressed for

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the 4 weeks duration between injection and culling. However, immunohistochemical studies of pituitary tumor sections revealed an increased and stable expression of menin, as assessed by a 3-point grading system, at 4 weeks in the mice treated with Men1.rAd5 (Fig. 4A) when compared with those treated with GFP.rAd5 or PBS (1.5 ± 0.13 in Men1.rAd5; 0.6 ± 0.32 in GFP.rAd5; and 0.5 ± 0.16 in PBS-only, *P* < 0.04). GFP expression occurred only in the pituitary tumors of Men1+/−/C0.
mice treated with GFP.rAd5 (Fig. 4B). Second, it is possible that the 4-week time interval posttreatment is too short to detect any significant changes in tumor volume. However, it was not possible to continue observing the mice for substantially longer periods because of deaths due to old age. Third, pituitary tumors of 4 different Men1+/− mouse models are known to be highly vascular and contain spaces that are filled with blood (29, 41–43), and the Men1.rAd5 will not have an effect on these nontumorous cells. Analysis of Men1+/− pituitary tumors revealed that 46 ± 3% of tumors were formed by blood-filled spaces, compared with 5 ± 1% in Men1+/− normal pituitaries (P < 0.00001; Fig. 3E and F). Therefore, the pituitary tumor cells formed approximately 55% of the total tumor volume indicating that approximately half of the tumor would be unaffected by gene replacement therapy. The Men1.rAd5 vector may have an antiproliferative effect on pituitary tumor cells, which form half of the tumor volume, but this effect may be too small to be detected by MRI.

To assess for alterations in pituitary tumor cell proliferation that may occur in response to the expression of menin by the Men1.rAd5 vector, we used IHC to identify cells labeled with BrdUrd, which is a thymidine analogue that is incorporated into DNA during the synthesis (S) phase of dividing cells (Fig. 4C). In addition, we assessed for apoptosis (Fig. 4D), as menin has been reported to promote apoptosis (44), and for an immune response (Fig. 4E), as the injection of an adenovirus may induce an immune reaction. Treatment with Men1.rAd5 resulted in a significant decrease in BrdUrd incorporation by pituitary tumor cells such that there was a 3-fold reduction in the daily proliferation rate when compared with treatment with GFP.rAd5 and PBS-only (0.45 ± 0.09% in Men1.rAd5 treated; 1.39 ± 0.13% in GFP.rAd5 treated; and 1.23 ± 0.10% in PBS treated, P < 0.0001; Fig. 4F). We further examined the proliferation rates of prolactin-expressing cells (Fig. 4C) in pituitary tumors, as the majority (>65%) of Men1+/− anterior pituitary tumor cells immunostain for prolactin (29). This revealed that treatment with Men1.rAd5 resulted in a 6-fold reduction in daily proliferation rates of prolactin-containing cells (Fig. 4F) when compared with treatment with GFP.rAd5 and PBS (0.19 ± 0.03% in Men1.rAd5 treated; 0.96 ± 0.13% in GFP.rAd5 treated; and 1.3 ± 0.13% in PBS-only treated, P < 0.00001). Menin expression by the Men1.rAd5 vector in anterior pituitary tumor cells was not associated with an increase in apoptosis, as was assessed using the TUNEL assay (Fig. 4D). Thus, the mean apoptotic rates in anterior pituitary tumors treated with Men1.rAd5, GFP.rAd5, or PBS were similar at 0.17 ± 0.06%, 0.19 ± 0.21%, and 0.20 ± 0.09%, respectively (P > 0.2).

Gene therapy using adenoviral vectors has been reported to cause cell-mediated immune responses, and we therefore assessed for a T-cell response by immunostaining for CD3, which is a membrane protein in T-cell receptor complexes (Fig. 4E). This revealed that intra-tumoral injection of rAd5 vectors was not associated with a cell-mediated immune response as the mean percentage of cells immunostaining with CD3 in anterior pituitary tumors treated with Men1.rAd5, GFP.rAd5, and PBS were similar at 0.17 ± 0.09%, 0.08 ± 0.02%, and 0.13 ± 0.08%, respectively (P > 0.3). Localized delivery of gene therapy using adenoviral vectors may potentially be associated with systemic spread, and to assess this we immunostained for GFP expression in the cerebrum, liver, lymph nodes, and adrenals of Men1+/− mice treated with GFP.rAd5. This revealed an absence of GFP expression in these tissues (data not shown), indicating that GFP.rAd5 had remained localized to the pituitary tumor (Fig. 4B) and not spread systemically.

Discussion

Our studies show that in vivo expression of menin by use of a recombinant adenoviral vector in pituitary adenomas of Men1+/− immunocompetent mice is effective in reducing tumor cell proliferation (Fig. 4) and is not associated with significant adverse effects or increased mortality (Fig. 2). These results are comparable to those of adenovirus-mediated retinoblastoma (Rb) gene therapy in pituitary melanotroph tumors in Rb-deficient mice, where intratumoral Rb cDNA delivery inhibited tumor growth (40). Thus, our results represent an important preclinical advance for the development of MEN1 gene therapy, which is likely to be of use in MEN1 patients for the treatment of anterior pituitary adenomas, foregut NETs, and pancreatic NETs; in non-MEN1 patients with foregut and pancreatic NETs as more than 40% of these have MEN1 somatic mutations and loss of menin expression (15); and in non-MEN1 patients with pancreatic ductal adenocarcinomas as approximately 45% of these have decreased expression of the MEN1 gene (45). In addition, MEN1 gene replacement therapy may have a possible wider use in other tumors that have other genetic abnormalities, as in vitro studies have reported that menin overexpression inhibits cell proliferation of non-MEN1 human endocrine pancreatic tumor cells (BON1 cell line; ref. 46), and of mouse RAS-transformed NIH3T3 MEFs (17). However, improvements in the delivery of the recombinant adenoviral vector, e.g., by a systemic route as opposed to direct intratumoral injection, which may need to be repeated, will be required to achieve better targeting of multiple NETs that occur in MEN1 patients, and to avoid the immune responses that are associated with multiple exposures to the adenovirus (47, 48). Such advances would likely represent important steps in providing effective treatments for NETs, particularly as metastatic gastroenteropancreatic NETs are a major determinant of mortality in MEN1 patients (1–5).

Our studies, which involved only one intratumoral injection, did not detect an immune response to the recombinant adenoviral vector in immunocompetent Men1+/− mice, and this contrasts with the results of other studies that have injected recombinant adenoviral vectors into the pituitaries of rats and sheep. The injection of recombinant adenoviral vectors to rat pituitaries was associated with an immune response, e.g., an increase in CD8 positive T cells, that peaked at 14 days postinjection and was resolved by 2 months (49), and the injection of recombinant adenoviral vector to ovine pituitaries resulted, within 7 days, in a significant hypophysectomy with lymphocytic infiltration (50). These differences in the immune reactions may be partly attributable to differences in: the immune responses of the 3 species studied: the recombinant adenoviral vectors; and the time points at which the analyses were undertaken. It is important to note that our
study only examined for an immune response at one time point, and further studies at additional time points will be required to determine the occurrence, if any, of an immune response. Indeed, more detailed studies of the innate and adaptive immune responses that are known to limit vector transduction efficiency and duration of transgene expression are required to facilitate improvements in MEN1 gene replacement therapy.

Menin expression, following intratumoral injection of the recombinant Men1 adenovirus, was not associated with increased apoptosis (Fig. 4). This is surprising, as in vitro studies in HEK293 cells and menin-null MEFs, have reported that menin expression induced apoptosis; thus, menin expression in menin-deficient pituitary tumor cells, in our study, would have been predicted to increase tumor cell apoptosis. However, the lack of an observed increase in apoptosis following in vivo menin expression in anterior pituitary tumor cells may in part be attributable to the differences in intrinsic oncogenic and apoptotic pathways of the cell types studied, and to the role of the surrounding cells of the matrix of the tumor in vivo, as well as the possibility that our analysis at 4 weeks may have missed any increase in apoptosis at earlier time points. Nevertheless, overall our results suggest that the predominant in vivo effect of menin loss is to increase cell proliferation rather than reduce apoptosis and, hence, MEN1 gene replacement is likely to lead to a decrease in tumor cell proliferation and growth.

In summary, our study has established the proof of concept for in vivo Men1 gene replacement in reducing cell proliferation of anterior pituitary adenomas, which develop in Men1+/− mice.

References

15. Lemos MC, Thakker RV. Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: G.V. Walls, M.C. Lemos, M. Javid, B. Harding, S. Piret, L. W. Seymour, R.V. Thakker

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