

Growth Hormone and Translational Research: From the 'Bench' to the 'Bedside'

John J. Kopchick^{1,2}, Darlene E. Berryman^{1,3}, Elahu Gosney¹, Edward O. List¹

Edison Biotechnology Institute¹, Ohio University, Athens; Department of Biomedical Sciences², Heritage College of Osteopathic Medicine, Ohio University, Athens; School of Applied Health Sciences and Wellness³, College of Health Sciences and Professions, Ohio University, Athens, OH, USA

INTRODUCTION

An understanding of the basic physiology of growth hormone (GH) as well as its fundamental role in various clinical conditions has been elucidated by the use of several strains of animals with altered GH action, including genetically modified mice. In this review, we will discuss three mouse lines that have extreme alterations in GH action and how one of these mouse strains was crucial in the discovery of a GH receptor antagonist (GHA) that has resulted in a drug (Somavert, Pfizer, New York, NY, USA) for the treatment of acromegaly. An overview of the basic phenotypes of these mice is provided in the Table 1 and a picture of them is shown in the Fig. 1.

1. GHR^{-/-} mice

In the mid 1990's, our laboratory generated growth hormone receptor (GHR) gene deleted mice (GHR^{-/-}) [1]. Because of their complete lack of GH induced signaling, these mice provide a valuable model to elucidate the numerous roles of GH during development and aging. Physiological changes between GHR^{-/-} mice and wild-type littermates are indicative of GH's actions. The GHR^{-/-} mice have been a productive tool to study GH action, resulting in more than 100 published studies (for a comprehensive review, see [2]).

GHR^{-/-} mice are dwarf with low levels of insulin-like growth factor-I (IGF-I) and concomitant increased levels of GH [1]. These mice have a number of striking features that have been recently reviewed [2]. First, they are extremely insulin sensitive [3-12]. Further, circulating levels of glucose and glucagon are decreased at a young age but normalize with advancing age [6,12]. The high insulin sensitiv-

ity observed in these mice highlights the anti-insulin activity of GH since the lack of GH signaling removes anti-insulin activity, resulting in improved insulin sensitivity. Second, these mice have a significantly higher percent body fat throughout their lifespan [3,13,14]. Thus, these mice provide a paradoxical situation of being both obese and insulin sensitive. Accordingly, leptin levels are elevated in GHR^{-/-} mice [5,12,13], which is consistent with their relative obesity. Interestingly, adiponectin levels, which are typically negatively correlated with obesity, are also elevated in GHR^{-/-} mice [12,13,15]. Thus, it appears that adiponectin is more strongly correlated with insulin sensitivity than obesity when an obese, insulin sensitive state co-exist. Another interesting feature of the increased adiposity in the GHR^{-/-} mice is that they store a disproportionate amount of fat in the subcutaneous white adipose tissue (WAT) depot. Because these mice have preferential enlargement of subcutaneous WAT and are extremely insulin sensitive, it is tempting to speculate that subcutaneous WAT is "healthier" than other adipose depots. This concept has been suggested by recent transplant and longevity studies [16,17].

One of the most remarkable findings of the GHR^{-/-} mice is that they live much longer than their wild-type littermate controls [8]. These mice have such an extension in lifespan that at the time this paper was written, they still hold the record for the longest-lived laboratory mouse (<http://www.methuselahfoundation.org/>). While it is not fully understood how the absence of GH action increases lifespan, GHR^{-/-} mice do show resistance to streptozotocin-induced kidney damage [18] and reduced incidence and delayed occurrence of fatal neoplastic diseases [19]. Further, it is important to remember that these mice are extremely insulin sensitive, which is theorized to account for some, if not most, of their lifespan extension.

Corresponding author: John J. Kopchick
Edison Biotechnology Institute and Department of Biomedical Sciences, College of Osteopathic Medicine, Ohio University, Athens, OH 45701, USA
Tel: +1-740-593-4534, Fax: +1-740-592-4795, E-mail: kopchick@ohio.edu

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Table 1. Phenotypic comparison of genetically modified mice with altered activity of GH

Mouse model	GH transgenic*	GHA transgenic†	GHR-/-‡
Level of GH signaling	↑↑	↓	None
Growth/Body weight	↑↑	↓	↓↓
Body composition (adult)			
% Lean mass	↑↑	↓	↓↓
% Fat mass	↓↓	↑↑	↑
Plasma levels			
GH	↑↑	↑	↑
IGF-1	↑↑	↓	↓↓
Glucose	↑/↔	↔	↓
Insulin	↑	↔	↓↓
Leptin	↓	↑↑	↑
Adiponectin	↓	↑	↑↑
Reproduction			
Time to reach sexual maturity	↓↓	ND	↑
Fertility	↓	↓	↓
Morbidity			
Tumor incidence	↑	ND	↓↓
Cardiac/Vascular deficits	↑	ND	↔
Kidney impairments	↑	↓	↓
Lifespan	↓	↔	↑

*Modified from [3,13,24,27,30,80-83]; †Modified from [13,51,53,64,84-86]; ‡As reviewed in [2]. GH, growth hormone; GHA, GH receptor antagonist; GHR, growth hormone receptor; IGF, insulin-like growth factor; ND, not determined.

For this reason, our laboratory and others are studying several new types of GHR gene disrupted mice in which the GHR gene is deleted from a specific tissue using cre/lox technology. Because insulin sensitivity is thought to play an important role in lifespan extension in GHR-/- mice, we have developed three mouse lines that lack GHR in three insulin sensitive tissues (liver, muscle, and fat). Analysis of how individual insulin sensitive tissues respond to the absence of GH signaling should further elucidate the role GH plays in glucose metabolism and the aging process.

2. Clinical: GHR-/- versus Laron syndrome (LS)

LS is an autosomal recessive disorder characterized by insensitivity to GH. LS is caused by mutations to genes that encode molecules in the GHR signaling pathway with the majority of cases occurring in the GHR gene itself [20,21]. Thus, the GHR-/- mouse is the equivalent of LS in humans. Comparisons between the GHR-/- mice and humans with LS have been extensively reviewed (for a more comprehensive review, see [22]). GHR-/- mice mimic the human condition in many ways. The most prominent similarities are decreased IGF-I levels and increased GH levels, an inability to respond to exogenous GH, growth retardation, delayed sexual matu-

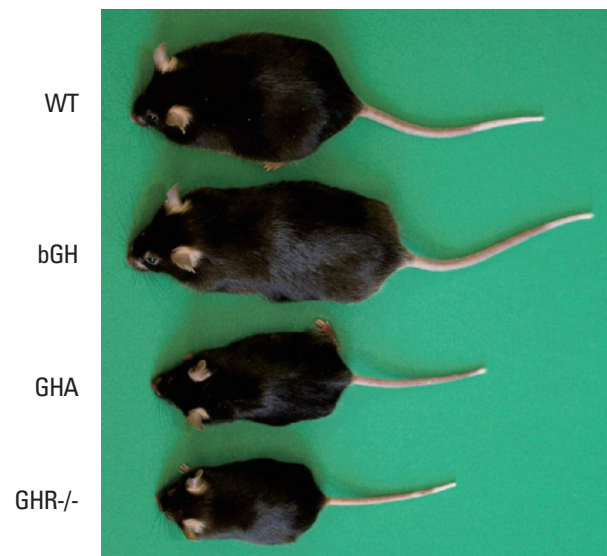


Fig. 1. An image of a wild-type (WT) mouse, a giant bovine growth hormone (bGH) transgenic mouse, a dwarf GH receptor antagonist (GHA) transgenic mouse and a dwarf growth hormone receptor (GHR) -/- mouse in the same genetic background is shown (C57BL/6 J). These mice represent normal, elevated, decreased and absent levels of GH action, respectively. Reprinted with permission from Berryman et al. Growth Horm IGF Res 21:113-123, 2011 [87].

ity, hypoglycemia at a young age, increased obesity with a disproportionate increase in subcutaneous adipose tissue, increased leptin and adiponectin, decreased muscle mass, decreased bone mineral density, decreased cranial bone size and a decreased incidence of cancer [22].

There are also some potential differences between GHR-/- mice and LS individuals that relate to glucose metabolism and longevity that appear to depend on which populations of LS individuals are studied. In certain cohorts of humans with LS, Laron and Kopchick [22] describe a tendency to become hyperinsulinemic with decreased insulin sensitivity with advancing age while the GHR-/- mice have low levels of insulin with increased insulin sensitivity at most ages. This difference in insulin sensitivity and insulin levels may explain the difference in lifespan as insulin sensitive GHR-/- mice have extended longevity, and insulin resistant humans with LS have normal lifespans [22]. However, in a separate cohort of LS individuals studied for 22 years, it was recently reported that those with LS have drastically reduced insulin concentrations, higher insulin sensitivity, with very low homeostasis model assessment of insulin resistance (HOMA-IR) scores, and a major reduction in cancer and diabetes [23]. Thus, depending on the population and associated environmental factors, it appears that some humans with LS actually do have improved glucose metabolism similar to the GHR-/- mice.

3. GH transgenic mice

Nearly thirty years ago, mice that expressed a rat GH transgene were first described [24]. Since then, multiple independent laboratories have generated similar mice using varying species of the GH transgene. These various transgenic animals have different levels of GH expression. It should be noted that because human GH can bind to both GH and prolactin receptors, overexpression of a human GH transgene results in a physiological state in which both GHRs and prolactin receptors are simultaneously activated. Since rat, bovine or ovine GH binds exclusively to the GHR, transgenic mice that express these GH genes have helped to elucidate and differentiate the specific role of GH action versus that of prolactin.

Overall, GH transgene expression in mice results in a fairly uniform phenotype. That is, female and male GH transgenic mice display accelerated growth starting at approximately three weeks of age and have a greater adult body weight as compared to control mice [24-27]. Elevated circulating GH also results in elevated plasma levels of IGF-I and hyperinsulinemia despite euglycemia [28]. Body composition studies in GH transgenic mice have been less consistent, but recent evidence suggests that the discrepancy may be due to the age at which the mice were examined [27]. By 4 to 6 months of age, adult male bovine GH (bGH) mice are lean with reductions in the weight of all adipose depots. They also have altered adipokine profiles with reduced leptin and adiponectin levels as well as increased resistin, tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) levels as compared to control mice [13,29]. In addition, these mice have been reported to be somewhat resistant to the effects of diet-induced obesity [3,30] while calorie restriction improves their glucose homeostatic and adipokine profiles [29]. Thus, GH has clear and poignant impacts on fat mass and adipokine levels in adult transgenic mouse models. Data in mice younger than 3 months of age have been conflicting, with some showing increases in fat mass [25,27,31,32] and others showing decreases [31]. Interestingly, many of the reported changes, such as body composition [27], show gender dependent effects with females having a less dramatic phenotype.

Lifespan of GH transgenic mice is drastically reduced (~50%) compared to non-transgenic controls [33,34]. The cause of the premature death is likely multifactorial. Specific pathological organ changes in these mice have been noted as a result of high GH levels. These changes include severe glomerulosclerosis and lipid accumulation in the kidney as well as enlargement of most GH sensitive tissues, with liver and spleen experiencing the greatest disproportionate

increase in size [35-41]. There is also evidence of impaired cardiac and vascular function [42-45], lipid abnormalities [46], kidney damage [47,48], and greater incidence and early onset of mammary tumors in at least hGH transgenic mice [49].

4. Clinical: GH transgenic mice versus acromegaly

Acromegaly is a disease characterized by a chronic excess of circulating GH usually caused by a GH-secreting pituitary adenoma, resulting in elevated levels of IGF-I. The condition is associated with considerable morbidity and increased mortality, as recently reviewed [50]. The outcome of this condition shares many features with GH transgenic mice. However, there are important distinctions that need to be considered. First, unlike the GH transgenic mice, the cause of the chronic excess of GH is most commonly due to autonomous GH secretion from pituitary adenomas and not by genetic alteration that result in ectopic GH secretion. Second, the level of GH secretion in GH transgenic mice varies, but often is significantly greater than what is observed with acromegaly. Finally, the transgenic mice have excess GH gene expression for most of their postnatal life most acromegalic individuals do not. Regardless, the transgenic mice and the acromegalic patient have similar phenotypes and thus provide a valuable tool to dissect the impact of GH excess on tissue physiology.

5. GHA transgenic mice

GHA transgenic mice express the GHA transgene. The transgene is a mutated bGH gene in which the codon for glycine at position 119 is replaced by arginine or lysine (G119K) [51-53]. Expression of these two mutated GH transgenes surprisingly results in reduced GH action and was later found to be a classic receptor antagonist [54].

The fortuitous discovery of this receptor antagonist is worthy of some discussion as it not only has provided a novel mouse strain with reduced GH action but also has resulted in a better understanding of the GH molecule as it interacts with its receptor. Most importantly, though, the discovery has resulted in the development of a novel drug, as will be discussed later in this review. The discovery of GHA helped establish that the stoichiometry of the GH:GHR is 1:2 [55,56]. Thus, GH was known to interact with two GHR monomers in order to initiate the signaling cascade, suggesting that two distinct regions of a single GH protein interact with each receptor monomer (it is now known that the GHR exists as a preformed homo-dimer). One of the regions in the GH molecule responsible for GH binding to GHR, referred to as site 1, has a higher affinity to

the GHR compared to the second site (Site 2); the amino acid residues important for Site 1 interaction had already been identified [56-61]. The second site remained obscure, but the likely region responsible for binding at Site 2 was thought to be located in helix 3 since this helix had previously been shown to have significant growth promoting activity [52,62]. After a more careful assessment of the structure of helix 3, we noted that this helix had an amphipathic configuration, with the exception of three amino acids. We hypothesized that the growth promoting activity of helix 3 could be further enhanced if the helix was modified to form a perfect amphipathic configuration. Thus, a mutant bGH gene was engineered that encoded three amino acid substitutions, converting the imperfect amphipathic 3rd helix to a perfect amphipathic helix (Q117L, G119R, and A122D). Unexpectedly, instead of enhancing the growth-promoting activity of GH, the perfect amphipathic amino acid substitutions in helix 3 antagonized the function of GH in both cell culture and whole animal systems [51,52,63]. After evaluating the relative importance of each single substitution, it was later confirmed that the G119R substitution was responsible for the GH inhibiting or antagonistic effect [53]. The mutation in this helix of GH allows GHA to bind properly at Site 1 to the preformed GHR dimer on the surface of target tissues, but prevents GHA from binding properly to the GHR homodimer at Site 2. Consequently, GHA binds to the preformed GHR dimer but fails to induce intracellular GHR signaling [51,53,63], and thus acts as a competitive inhibitor of endogenous GH. Mice that express the GHA transgene with the single G119K or G119R mutation have been extremely useful in understanding the potential impact of GH deficiency as well as laid the foundation for developing the GHA into a pharmaceutical agent, as will be discussed later in this review.

The overall phenotype of GHA mice is generally intermediate to that of control and GHR^{-/-} mice. That is, GHA mice have an overall dwarf phenotype [51,53,63], but not as severe as is seen with the GHR^{-/-} mice [8]. Interestingly, by approximately 11 months of age, the body weight of male GHA mice gradually catches up with controls [8]. Data from our laboratory show that the eventual weight gain is caused by increased adiposity without major gains in lean mass (Berryman et al., submitted), indicating that these mice remain dwarf but become markedly obese with advancing age. Other reports have shown young GHA mice to be obese [13,64] with specific enlargement of the subcutaneous fat pad, a trait shared with GHR^{-/-} mice.

Several serum factors have been assessed in these mice. Male GHA

mice have a ~80% reduction in serum IGF-I and a 30% reduction in IGFBP-3 when compared to controls [8]. Glucose and insulin levels appear to be somewhat dependent on age. That is, young male GHA mice tend to have lower levels glucose and insulin [8,64] but higher levels with advancing age [8] (Berryman et al., submitted). Thus, the effect of age seems to be a significant factor for some of these serum parameters. Finally, adipokine levels have been assessed in several studies. Similar to GHR^{-/-} mice, young, obese GHA male mice display elevated plasma leptin levels [13,64] as well as elevated adiponectin levels [13].

Longevity in GHA mice has only been reported in a single study [8]. Although there was a tendency for GHA mice to live longer than littermate controls, the difference did not reach statistical significance. Interestingly, the trend towards an increase in lifespan was more notable for the female GHA mice (839 ± 25 days) as compared to control female mice (771 ± 26 days). Additional studies including larger numbers of mice are needed to confirm these results. Of note, GHA mice offer an interesting exception to other mouse lines with a decrease in GH signaling, such as the GHR^{-/-} mice. While GHA mice share many features with GHR^{-/-} mice, they do not exhibit major improvements in lifespan. Thus, GHA mice provide a unique opportunity to determine key traits necessary for lifespan extension. Importantly, the GHA mouse is a more clinically relevant system than that of the long-lived GHR^{-/-} mouse, since repression of GH action is attainable therapeutically with the use of a GHA (Somavert) whereas total repression of GH action is not.

6. Development and design of pegvisomant

The mutation of the bGH gene, namely (G119R or K), led to the discovery of the GHA and proved to be an effective antagonist of GHR *in vitro* and *in vivo*. Once this was known, attention shifted to whether a comparable mutation in human GH (G120K) would result in an effective antagonist for clinical indications. In order to become an effective pharmaceutical agent, the antagonist would have to overcome at least one major physiological hurdle; that is, the short serum half life of GH and G120K. Endogenous GH has a half life of approximately 15 minutes [65], with G120K also exhibiting a short serum half life. Conjugation of 5-kDa polyethylene glycol (PEG) moieties to GH had been known to increase its serum half life [66]; thus, pegylation was investigated as a method to improve the pharmacokinetics of GHA. PEG binds to primary amine groups, therefore attaching to lysine residues and the N-terminus. The benefits of pegylation to the pharmacokinetics of a peptide drug

must be balanced with the steric hindrance caused by the addition of the PEG molecules to regions of the protein that are important for receptor binding. Two lysines are present in a region of GH critical for binding to the GHR receptor at site 1. In order to preserve the binding affinity of PEG-G120K for GHR, these lysines were mutated to other amino acids (K168A and K172R) so that they do not become pegylated, which could reduce receptor binding due to steric hindrance. There are also six other site 1 mutations (H18D, H21N, R167N, D171S, E174S and I179T) shown to increase the binding affinity at site 1 [56] that have been incorporated into GHA to increase the potency of the drug [67]. The final recombinant protein, a drug named pegvisomant (Somavert), consists of G120K with the eight site 1 mutations described above along with 4-5 conjugated PEG-5000 moieties. While pegvisomant has a 20-fold reduced affinity for cell-surface GHR due to pegylation [67], it is an effective antagonist due to an increase in serum half life of approximately 100 hours, a characteristic conferred by pegylation [68]. An additional benefit of the site 1 mutations is that they abolish the ability of the molecule to bind to the prolactin receptor [69], thus reducing off-target pharmacologic effects.

7. Pegvisomant characterization in rodents and primates

In rats, human GHA (hGHA) fails to antagonize GHR activity in GH-deficient dwarf rats when co-infused with GH [70]. In fact, hGHA is paradoxically observed to act in an additive manner with GH in promoting skeletal growth in these animals, possibly due to binding of G120R to the prolactin receptor [70]. In mice, pegvisomant can reduce IGF-I levels by up to 70%, but only at a dose 10-20 times that normally given to treat acromegaly in humans [71]. A bovine version of the antagonist (G119R) expressed as a transgene in mice (GHA mice), as noted above, is similarly effective at inhibiting GHR activity in mice.

Prior to clinical trials, two studies conducted in rhesus monkeys showed the effectiveness and safety of pegvisomant in primates. In the first study, a weekly bolus of 1 mg/kg of pegvisomant was injected subcutaneously into ovariectomized monkeys [72]. By day 3 post-injection, serum IGF-I levels were 61% lower than controls, with a maximal depression observed at day 10. At 14 days post-injection, IGF-I levels normalized to that of controls. In a follow-up study, five ovariectomized monkeys were treated with pegvisomant [73]. Animals were given the same dose of pegvisomant as in the first study. IGF-I levels began to fall within 24 hours of pegvisomant administration and continued to decline unto day 5, when levels had dropped

78%. These primate studies showed pegvisomant to be a well tolerated inhibitor of GH action.

8. Clinical trials

Once pegvisomant was shown to be safe in primates and an effective suppressor of IGF-I, clinical trials in humans were undertaken. In a phase I, placebo-controlled, single rising-dose study, 36 healthy young men received subcutaneous doses of pegvisomant at 0.03, 0.1, 0.3 or 1 mg/kg [74]. There were no serious adverse events from any of the pegvisomant doses. A dose dependent decrease in IGF-I levels was observed for the 0.3 and 1.0 mg/kg doses, with maximum suppression observed on day 5 post-injection with 1 mg/kg pegvisomant, at which time IGF-I was nearly half baseline levels. There were no substantial changes in circulating GH levels at any of the doses given; however, an extremely small yet significant rise in mean GH levels was observed for the 1 mg/kg dose in one of two GH quantification assays.

The first study reporting treatment of patients with pegvisomant was presented at The Endocrine Society's Annual Meeting in 1998 [75]. In this double blind, placebo controlled phase II study, 46 patients with acromegaly were given placebo or one of two doses of pegvisomant. Nearly two thirds of the patients had previously received surgery, radiotherapy and octreotide treatment. All patients in this study were withdrawn from any other medical treatment for three weeks prior to receiving pegvisomant. Patients receiving 30 mg/wk of pegvisomant had a 16% reduction in total IGF-I levels, while those on 80 mg/wk had a 31% drop in total IGF-I after six weeks of treatment. Free IGF-I decreased even further, with a 47% drop in the 80 mg/wk group after six weeks of treatment. Pegvisomant was well tolerated, and no patients were dropped due to adverse events.

The phase III clinical trial established the short term safety and efficacy of pegvisomant in treating patients with acromegaly [76]. In this randomized, double-blind, placebo-controlled study of 112 patients with acromegaly, daily doses of pegvisomant were administered subcutaneously for 12 weeks. Patients were selected by withdrawal of other drugs and a serum IGF-I level greater than 1.3 times the upper level of the normal age-adjusted range. Patients were randomly selected to receive a daily dose of 10, 15, or 20 mg of pegvisomant or placebo. In all three treatment groups, IGF-I, IGFBP-3, and acid-labile subunit decreased in a dose-dependent fashion. The number of patients who achieved normal IGF-I levels increased with dosage, ranging from 38% at 10 mg/day up to 82% at 20 mg/day.

Normalization of IGF-I levels was achieved rapidly, with 75% of the maximal reductions occurring within the first two weeks of treatment. Even though three patients at the 20 mg/day dose did not achieve normal IGF-I levels, they all showed substantial decreases in IGF-I levels. Various symptoms in the treatment groups showed improvement, including decreases in soft tissue swelling, excessive perspiration and fatigue. There was a dose-dependent increase in serum GH levels, coinciding with the decrease in serum IGF-I levels, reflecting the negative feedback loop that regulates GH secretion from the pituitary. The drug was well tolerated, with only one patient classified as experiencing a serious adverse reaction. This patient had elevated levels of alanine and aspartate aminotransferases in the serum under treatment that returned to normal within eight weeks of discontinuation but were elevated once again after going back on pegvisomant treatment for four weeks.

9. Initial long-term pegvisomant trial

While the phase III trial showed great promise for pegvisomant, questions regarding the safety and efficacy of long-term use remained. A cohort of 160 patients treated for an average of 425 days was closely analyzed to answer questions not sufficiently addressed by earlier trials and to determine the suitability of pegvisomant for acromegaly treatment [77]. Patients included in this study were required to discontinue other medical interventions and have a serum IGF-I level at least 1.3 times the upper limit of the normal range. Patients were started on a dose of 10 mg/day of pegvisomant with a titration up or down of 5 mg/day until IGF-I level normalization, with a maximum dose of 40 mg/day. For data analysis, patients were divided into groups receiving pegvisomant for 6, 12, or 18 months, with all patients in the 12 month group also included in the 6 month cohort and all 18 month patients included in both other groups. Impressively, IGF-I levels were normalized in 97% of patients ($n = 90$) treated with pegvisomant for 12 months or more. Serum GH levels were increased in all patient groups, rising 12.5 $\mu\text{g/L}$ in the 6 and 12 month cohorts and by 14.2 $\mu\text{g/L}$ in the 18 month group. In patients withdrawn from pegvisomant and not placed on alternative medical therapy, GH levels returned to baseline levels within 30 days, supporting the data showing that pegvisomant treatment is responsible for the elevated GH levels. Antibodies to pegvisomant were detected in roughly 17% of patients, although none showed signs of tachyphylaxis. Parameters of insulin resistance, a common side effect of acromegaly, showed improvement with decreases in both insulin and glucose concentrations in all three treatment groups.

This is particularly important in light of the fact that nearly one third of untreated patients with acromegaly develop type 2 diabetes. None of the patients included in this study were diagnosed with diabetes at baseline. The fact that pegvisomant treatment improved these important metabolic parameters in acromegalic individuals, who are especially vulnerable to the development of diabetes and who may be exhibiting signs of pre-diabetes, is one of the benefits not observed with somatostatin analog use [78]. Magnetic resonance imaging scans were analyzed to determine the effect of pegvisomant treatment on pituitary tumor growth. There was no change in mean tumor volume for the 131 patients for which scans were available. However, 53 patients that were never treated with radiation therapy showed a mean tumor volume increase of 0.103 cm^3 over an average treatment duration of approximately 10 months. No association between tumor volume changes and pegvisomant treatment duration was observed. In two patients, tumor growth required treatment to reduce tumor volume; however, the cause of tumor growth in these patients was not clear. Later studies with more patients and longer-term follow-up, as discussed below, have shown that tumor progression is a rare event in patients treated with pegvisomant.

10. ACROSTUDY

Due to the fact that pegvisomant was a drug with a new mechanism of action, the regulatory agencies in the USA and Europe required an ongoing observational survey to determine the long-term efficacy and safety of the new therapeutic. This study was started in 2004 and is ongoing under the name ACROSTUDY [79] and has/ is being carried out by Pfizer Inc.

As of February 2009, more than 792 patients had been included in the study with a mean of 3.3 years of pegvisomant treatment. A vast majority of patients were receiving pegvisomant daily, and 67% were not taking other medical therapies for acromegaly. Average IGF-I levels dropped from 518 ng/mL to 277 ng/mL after 12 months of treatment. The proportion of patients who achieved IGF-I levels within the normal, age-adjusted range was relatively constant at 62% regardless of the length of treatment. Those patients taking other medical therapies in combination with pegvisomant had similar rates of IGF-I normalization as those on monotherapy. This finding was unexpected given that 97% of patients achieved normalized IGF-I levels in an earlier open-label extension study, as discussed above [77]. The biggest reason for this discrepancy is likely a failure of physicians to increase the dose of pegvisomant in patients who

do not show IGF-I normalization [79]. In fact, four out of five patients with higher than normal IGF-I levels after three years of treatment remained on a dose of 20 mg/day or lower. Careful dose titration by the treating physician would likely achieve IGF-I normalization in a vast majority of patients.

Adverse events were reported in 142 patients, 56 of which were attributed to pegvisomant therapy. The most common adverse event reported was abnormal liver enzymes, appearing in 29 patients (3.7%). Liver enzyme levels were documented to return to normal in 10 of these patients, half of which remained on pegvisomant and several of whom were restarted on pegvisomant. There were 56 reported serious adverse events in a total of 46 patients, 13 of which were attributed to pegvisomant, with 9 due to elevated liver enzymes. Importantly, no patients treated with pegvisomant show any evidence of sustained liver damage.

There were three patients with pituitary tumor growth reported as a serious adverse event. Pre-treatment and post-treatment magnetic resonance imaging scans were available for 411 patients to investigate what effect, if any, pegvisomant has on pituitary tumor volume. Of these, 70 patients had a change in tumor size during treatment, with 31 showing a decrease and 22 showing an increase. Of those with lower tumor volume on pegvisomant, 26 had been either treated with radiotherapy previously, or were on combination therapy (either somatostatin analogue, dopamine agonist or both), which is likely the cause of tumor shrinkage. Of those with an increase in tumor volume, 6 had previously received radiation therapy and two had discontinued treatment with a somatostatin analogue. In two of the 22 patients, tumor growth was observed before pegvisomant treatment began. In 11 patients, additional review was unable to confirm a suspected increase in tumor size by the investigator.

As ACROSTUDY continues to accumulate patient data, it will not only continue to bring a greater understanding of the safety and efficacy of pegvisomant, but also help to improve the lives of those with acromegaly by providing a great wealth of knowledge to investigators and physicians that can guide best treatment practices into the future.

CONCLUSION

By the use of mouse strains generated in our laboratory that have either enhanced or repressed GH action, much has been elucidated about the *in vivo* functions of GH. Importantly, these studies have

resulted in the discovery of a drug, Somavert that is useful in the treatment of acromegaly. This work was truly translational in that it evolved from basic discoveries at the 'bench' to *in vivo* discoveries in the 'mouse' to the human 'bedside.' Additionally, one of these mouse strains (GHR^{-/-}) is the longest lived laboratory mouse and has been widely used in aging studies. We are very proud of these discoveries and look forward to future advances in the GH arena.

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