



2 PERSPECTIVES ON *Pharmacogenomics & Breast Cancer*

INTRODUCTION

Breast cancer is a formidable foe. Although chemotherapy has come a long way, a subset of patients becomes resistant to certain therapies, making their recovery more of a challenge. Researchers now have a new weapon in their arsenal—pharmacogenomics—to help them stay one step ahead of this shifting enemy. Recognizing that no one drug treats all, these studies entail looking at patients' genetic variants and their known interactions with drug efficacy or toxicity, in the hopes of matching the right drug to the right patient. Here, a clinical scientist and a basic researcher discuss this field of personalized medicine, focusing on patient responses to tamoxifen and aromatase inhibitors (AIs).

CLINICAL SCIENTIST PERSPECTIVE

► By Matthew P. Goetz, M.D.

Dr. Goetz is associate professor of oncology and pharmacology at Mayo Clinic in Rochester, Minn.



Clinical Highlights

- AIs are superior to tamoxifen for treating postmenopausal, ER+ breast cancer.
- The pharmacogenomics of tamoxifen and AIs may facilitate individualized therapy, because these drugs exhibit different mechanisms of antiestrogenic activity and undergo different metabolic routes of elimination and/or activation.
- Endoxifen is a potent tamoxifen metabolite; endoxifen concentrations are associated with *CYP2D6* genetic polymorphisms and drug-induced inhibition of the *CYP2D6* enzyme.
- Controversy exists regarding the association between *CYP2D6* enzyme deficiency and breast cancer recurrence in tamoxifen-treated women.
- National Cancer Institute's development of endoxifen to treat ER+ breast cancer is ongoing, with plans to commence clinical studies in early 2011.

Endocrine Therapy of Breast Cancer in 2010

Estrogen, through binding to either estrogen receptor (ER) α or β , regulates a wide variety of cellular effects and physiological conditions, including breast cancer cell proliferation. Selective estrogen receptor modulators (SERMs), such as tamoxifen, disrupt ER activity in cells by blocking estrogen's binding to the receptors.

AIs were developed based on the hypothesis that reducing the ligand responsible for stimulating breast cancer growth would offer superior breast cancer treatment. Randomized "head-to-head" studies comparing tamoxifen and AIs in the adjuvant treatment of this cancer demonstrated a significant improvement in disease-free survival in favor of AIs.¹ Although the Oxford overview showed an overall survival advantage in favor of the sequencing strategy (tamoxifen followed by AI),¹ many clinicians still recommend up-front AI use over tamoxifen. This preference is not only because of the differences in disease recurrence, but also due to rare but life-threatening side effects associated with tamoxifen use such as deep venous thrombosis.

Pharmacogenomics

A common focus in the area of cancer biomarkers has been somatic or tumor genetic variation and its association with disease recurrence. However, an emerging study area is the

pharmacogenomics of drug therapy. "Pharmacogenomics" generally refers to the role of germline genetic variation and its effects on drug metabolism, uptake, and distribution, and on drug targets. Even though the final common pathway of both SERMs and AIs is to disrupt estrogen signaling, these drug classes have vastly different metabolic routes of elimination and/or activation, and each metabolic step is under unique genetic control. In this article, the main focus is the pharmacogenomics of tamoxifen.

Tamoxifen Up Close

Tamoxifen's pharmacology is complex; it is a weak anti-estrogen that is extensively metabolized, and many of its metabolites show similar or more potent anti-estrogenic activity. Furthermore, tamoxifen can exhibit either anti- or pro-estrogenic properties, depending on the target tissue and the presence or absence of co-activators/co-repressors.

The potent tamoxifen metabolite most commonly used as an in vitro surrogate for tamoxifen activity is 4-hydroxy-tamoxifen (4-OH-tam), despite the fact that plasma concentrations of 4-OH-tam observed in humans (5 nM–10 nM) show minimal in vitro activity. Another hydroxylated metabolite, 4-OH-*N*-desmethyl-tamoxifen (endoxifen), like 4-OH-tam, is approximately 100-fold more potent an ER antagonist than tamoxifen itself;² however, human plasma concentrations of this metabolite are up to 10-fold higher than of 4-OH-tam.³ Although initial studies suggested that both 4-OH-tam and endoxifen had similar potency in ER binding affinity,⁴ in suppression of estradiol-stimulated cell proliferation,² and in gene expression,⁴ recent data suggest that the mechanism of action of these two SERMs differs, given that endoxifen uniquely targets ER α for proteasomal degradation in some model systems.⁵

Endoxifen is formed by the *CYP2D6*-mediated oxidation of *N*-desmethyl tamoxifen.^{2,6} Common genetic variants in *CYP2D6* and/or drug-induced inhibition of *CYP2D6* enzyme activity significantly reduces endoxifen concentrations in tamoxifen-treated humans.³ In an in vitro model system in which breast cancer cells are exposed to clinically relevant concentrations of tamoxifen and its major metabolites, endoxifen's effect on ER α degradation, transcription, and inhibition of proliferation is concentration-dependent. These assays revealed minimal effects at low endoxifen concentrations in human *CYP2D6* poor metabolizers (20 nmol/L), but significantly larger effects at concentrations in human *CYP2D6* intermediate (40 nmol/L–60 nmol/L) and extensive metabolizers (80 nmol/L–100 nmol/L).⁵ These in vitro data support the hypothesis that variation in the human concentrations of tamoxifen and its metabolites may account for de novo and/or acquired resistance to tamoxifen.

Challenges Studying Oral Drug Therapy Pharmacology

Following an initial study demonstrating an association between *CYP2D6* genetic variation and tamoxifen treatment

outcome,⁷ an updated analysis of more than 1,300 patients from Germany and the United States showed that CYP2D6-poor metabolizers had an approximately 2-fold higher risk of disease recurrence than CYP2D6-extensive metabolizers.⁸ However, several other studies have not confirmed this association.⁹ Similarly, extensive heterogeneity has been observed in studies evaluating drugs that alter CYP2D6 enzyme activity and tamoxifen response. A recent large population study evaluating women who received adjuvant tamoxifen and paroxetine, a CYP2D6 inhibitor, demonstrated a step-wise increase in breast cancer mortality in these patients, based on duration of paroxetine exposure.¹⁰ In contrast, other studies evaluating the association between CYP2D6 inhibitor use and tamoxifen treatment outcome have not shown an association with disease recurrence.^{11, 12}

Why have studies of tamoxifen pharmacogenomics yielded contradictory results? One potential reason is study design. Unlike “disease risk genomics,” which are most commonly studied retrospectively in populations where details of their exposure to anti-cancer therapy may be sketchy, the study of biomarkers that influence drug exposure must control for critical variables affecting delivery of the drug and/or metabolites, with additional control for other treatments known to alter the recurrence rate (e.g., surgery, radiation, chemotherapy, and other hormonal therapies). One of the most important variables affecting drug exposure is adherence. Notably, sub-optimal adherence to drug therapy has been observed in up to 40% of individuals taking adjuvant hormonal therapy and has been linked to higher rates of death.¹³ After publication of data suggesting that the *CYP2D6* genotype may be associated with hot flashes,⁷ one study demonstrated that patients with higher CYP2D6 enzyme activity (e.g., extensive metabolizers) had higher rates of drug discontinuation.¹⁴

Additional variables affecting drug exposure include dose, schedule, duration of drug therapy (e.g., 2 years vs. 5 years), and other environmental factors (e.g., drug-to-drug interactions) that alter enzyme activity and thus drug or metabolite exposure. For these reasons, the outcomes of studies analyzing the role of *CYP2D6* polymorphisms in the context of prospective tamoxifen and AI studies (e.g., BIG 1-98 and ABCSG 8) are eagerly awaited.

Endoxifen vs. ER+ Breast Cancer

Based on the extensive preclinical and clinical findings regarding endoxifen’s importance, the National Cancer Institute, in collaboration with investigators at Mayo Clinic, is currently developing endoxifen as a primary drug for treatment of ER+ breast cancer, including producing clinical grade endoxifen hydrochloride and preclinical toxicology/pharmacology for Investigational New Drug application submission. The first-in-human studies are expected to commence in 2011. Given that exposure to endoxifen in tamoxifen-treated patients is limited, there is great interest in determining whether the achievement

of higher endoxifen concentrations results in superior clinical outcomes, especially in patients expected to have endocrine-sensitive breast cancer who previously have progressed on tamoxifen.

BASIC SCIENTIST PERSPECTIVE

► By Steffi Oesterreich, Ph.D.

Dr. Oesterreich is a visiting professor at the Department of Pharmacology and Chemical Biology at the University of Pittsburgh School of Medicine, Penn., and the University of Pittsburgh Cancer Institute.



Basic Science Highlights

- “Pharmacogenomics” is the study of the “influence of genetic variations on drug responses in patients that is accomplished by correlating gene expression or single nucleotide polymorphisms (SNPs) with a drug’s efficacy and/or toxicity.”
- The possible impact of SNPs on drug metabolizing enzymes (e.g., CYP2D6) and drug transporter genes in response to chemo- and endocrine-therapies is of great interest.
- Associations may exist among response to novel targeted therapies and variants in the genes for key signaling proteins.
- Promising new data are emerging on the role of aromatase gene polymorphisms and response to AIs.

Pharmacogenomics in Breast Cancer Treatment

Pharmacogenomics is the study of the influence of genetic variation on drug responses in patients through correlation of gene expression or single nucleotide polymorphisms (SNPs) with a drug’s efficacy or toxicity. The goal of this approach is to personalize medicine. Breast cancer patients exhibit substantial inter-individual variability in treatment response as well as susceptibility to toxic and adverse effects related to therapy. Chemo-, endocrine, and targeted therapies for breast cancer are all affected by the genetic make-up of both the individual and the tumor. For example, somatic genetic changes within the tumor, such as amplification of the human epidermal growth factor receptor 2 (*ErbB2/Her2*) gene, can predict response to Herceptin (trastuzumab) therapy. However, there is limited knowledge about the power of germline genetic variation to predict the response to specific therapies.

The study of the pharmacogenomics of chemotherapy response has mainly examined metabolizing enzymes (e.g.,

cytochrome P450 and UGT enzymes) and drug transporters such as ATP-binding cassette (ABC). As an example, SNPs of UGT1A1, which is responsible for conjugation and inactivation of SN38, the active metabolite of irinotecan, have been linked to the drug's increased toxicity from decreased SN38 clearance. Polymorphisms in the transporter ABCB1 have been associated with inter-patient differences in response to chemotherapy (and hormonal therapy). However, a recent review concluded that knowledge is limited regarding the pharmacogenomics of chemotherapy response due to the limited number of candidate genes for study, the lack of reproducibility between studies, and the fact that chemotherapy is mostly given as combination therapy.¹ Novel approaches are therefore needed to make substantial progress in this area, such as the use of a panel of human lymphoblastoid cell lines to identify SNPs in candidate genes that correlate with response to chemotherapy,² as well as more intense data sharing through the formation of large consortia (see sidebar).

Currently, little is known about the polymorphisms that may predict response to novel targeted therapies such as those aimed at ErbB2. However, given that somatic mutations in EGFR predict response to its inhibitors in lung cancer³ and that FGFR polymorphisms are associated with breast cancer risk and outcome, we can expect to see more on the role of growth factor receptor SNPs and response to targeted therapies.

Genetic Variants and Endocrine Treatment

Breast cancer patients with ER+ tumors are treated with the nonsteroidal antagonist tamoxifen, and/or AIs. The latter blocks conversion of androgens to estrogens, as discussed below.

Tamoxifen. Tamoxifen is a prodrug metabolized by CYP P450 enzymes to the more potent 4-OH-tam and endoxifen compounds (please see Clinical Scientist's perspective for more details).

SNPs in *CYP2D6* that are associated with lower or no activity of the enzyme reduce endoxifen concentrations. Although controversial, evidence exists that such SNPs are associated with a weaker response to tamoxifen therapy. Because tamoxifen is given at a dose thought sufficient to saturate ER binding, models predict that the reported changes in endoxifen concentrations will not significantly affect ER binding by 4-OH-tam.⁴ However, if endoxifen's antiestrogenic activity occurs through mechanisms distinct from those of tamoxifen (e.g., if endoxifen binding results in a different structure of ER and alters subsequent interaction with coregulator proteins), small changes in endoxifen concentration could bring important changes in ER activity. Indeed, a recent study showed that endoxifen degrades ER, in contrast to 4-OH-tam which increases ER stability.⁵ However, other studies have shown little or no significant differences between 4-OH-tam and endoxifen on ER activ-

Pharmacogenomics at the NIH

Pharmacogenomics researchers seeking data for their projects can call off their widespread search. The National Institutes of Health (NIH) has boosted funding for two key resources for this field: a one-stop shop called the Pharmacogenomics Knowledge Base (PharmGKB) and the Pharmacogenomics Research Network (PGRN).

Begun in 2000 to catalog links between human genetic variation and drug responses, the PharmGKB Web site (www.pharmgkb.org) is now a centralized hub that collects, analyzes, and integrates data from national and international consortia. All gathered information is carefully annotated and cross-referenced with related research data. PharmGKB will receive \$15 million from NIH and will develop tools to extract information from biomedical literature and databases, analyze the genomes of additional individuals, and develop guidelines for doctors on using genetic tests to customize dosages when prescribing certain medicines.

PharmGKB is part of a broader NIH pharmacogenomics initiative that includes individual research projects and a nationwide research consortium, the NIH Pharmacogenomics Research Network (PGRN). The NIH plans to spend \$161.3 million to expand PGRN. Also launched in 2000, the PGRN has already identified gene variants linked to responses to medicines for conditions such as certain cancers and heart disease.

"Through these studies, we are moving closer to the goal of using genetic information to help prescribe the safest, most effective medicine for each patient," said NIH director Francis S. Collins, M.D., Ph.D. "There has never been a better time to propel the field of pharmacogenomics."

ity, as measured by proliferation⁶ and gene expression assays.⁷ Additional studies in cell lines and animal models are required to understand differences in the mechanism of action of antiestrogenic drugs.

Clinical studies assessing *CYP2D6* variants and breast cancer outcome are inconsistent. One striking observation is that the range of endoxifen concentrations varies widely, even among women with similar *CYP2D6* genotypes. It is therefore critical to explore further, in both mechanistic and epidemiological studies, how other enzymes and their variants influence tamoxifen metabolism, endoxifen levels, and treatment response. Candidate genes are the UGT family members (e.g., *UGT2B7* and *UGT1A8*), which mediate glucuronidation of tamoxifen and its metabolites and enhance their excretion in the urine and bile, thus potentially decreasing their activity.⁸

Aromatase inhibitors. As is the case with tamoxifen, patient response to AI therapy varies widely. One study recently identified two tightly linked SNPs in the 5' flanking region (exon 1.1) of the aromatase gene *CYP19* that are associated with higher baseline aromatase activity and greater change in estrone levels after AI therapy.⁹ These SNPs might alter *CYP19* gene expression. Further analysis of these interesting polymorphisms that measures AIs effect on gene expression, coupled with detailed functional studies, will require unique and large patient sample collections, including plasma and tumor hormone levels, germline DNA, and tumor tissue.

Challenges and Promises

In summary, most studies linking candidate gene polymorphisms with breast cancer treatment response have produced conflicting results. Future research will require more in-depth analysis of SNPs, most likely using the ability of next-generation sequencing to identify novel low-frequency SNPs, and will require large patient cohorts. With the rapid expansion in sequencing capacity and falling costs, it is likely that an explosion of data on genotypes and phenotypes will occur. Intense bioinformatics will be needed to understand the role of single and multiple SNPs in these processes. Advances may come from specifically focusing on panels of SNPs in pathways involved in determining response to a specific drug. For example, one might envision the prediction of endocrine therapy response from a combination of SNPs in the genes for metabolizing enzymes, ERs, and co-regulator proteins. Focusing on the identification and characterization of functional SNPs should increase the likelihood that pharmacogenomics will be incorporated into the clinical treatment of breast cancer. ■

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One additional case of thyroid C-cell hyperplasia in a Victoza®-treated patient and 1 case of MTC in a comparator-treated patient have subsequently been reported. This comparator-treated patient with MTC had pre-treatment serum calcitonin concentrations >1000 ng/L suggesting pre-existing disease. All of these cases were diagnosed after thyroidectomy, which was prompted by abnormal results on routine, protocol-specified measurements of serum calcitonin. Four of the five liraglutide-treated patients had elevated calcitonin concentrations at baseline and throughout the trial. One liraglutide and one non-liraglutide-treated patient developed elevated calcitonin concentrations while on treatment. Calcitonin, a biological marker of MTC, was measured throughout the clinical development program. The serum calcitonin assay used in the Victoza® clinical trials had a lower limit of quantification (LLOQ) of 0.7 ng/L and the upper limit of the reference range was 5.0 ng/L for women and 8.4 ng/L for men. At Weeks 26 and 52 in the clinical trials, adjusted mean serum calcitonin concentrations were higher in Victoza®-treated patients compared to placebo-treated patients but not compared to patients receiving active comparator. At these timepoints, the adjusted mean serum calcitonin values (~1.0 ng/L) were just above the LLOQ with between-group differences in adjusted mean serum calcitonin values of approximately 0.1 ng/L or less. Among patients with pre-treatment serum calcitonin below the upper limit of the reference range, shifts to above the upper limit of the reference range which persisted in subsequent measurements occurred most frequently among patients treated with Victoza® 1.8 mg/day. In trials with on-treatment serum calcitonin measurements out to 5-6 months, 1.9% of patients treated with Victoza® 1.8 mg/day developed new and persistent calcitonin elevations above the upper limit of the reference range compared to 0.8-1.1% of patients treated with control medication or the 0.6 and 1.2 mg doses of Victoza®. In trials with on-treatment serum calcitonin measurements out to 12 months, 1.3% of patients treated with Victoza® 1.8 mg/day had new and persistent elevations of calcitonin from below or within the reference range to above the upper limit of the reference range, compared to 0.6%, 0% and 1.0% of patients treated with Victoza® 1.2 mg, placebo and active control, respectively. Otherwise, Victoza® did not produce consistent dose-dependent or time-dependent increases in serum calcitonin. Patients with MTC usually have calcitonin values >50 ng/L. In Victoza® clinical trials, among patients with pre-treatment serum calcitonin <50 ng/L, one Victoza®-treated patient and no comparator-treated patients developed serum calcitonin >50 ng/L. The Victoza®-treated patient who developed serum calcitonin >50 ng/L had an elevated pre-treatment serum calcitonin of 10.7 ng/L that increased to 30.7 ng/L at Week 12 and 53.5 ng/L at the end of the 6-month trial. Follow-up serum calcitonin was 22.3 ng/L more than 2.5 years after the last dose of Victoza®. The largest increase in serum calcitonin in a comparator-treated patient was seen with glimepiride in a patient whose serum calcitonin increased from 19.3 ng/L at baseline to 44.8 ng/L at Week 65 and 38.1 ng/L at Week 104. Among patients who began with serum calcitonin <20 ng/L, calcitonin elevations to >20 ng/L occurred in 0.7% of Victoza®-treated patients, 0.3% of placebo-treated patients, and 0.5% of active-comparator-treated patients, with an incidence of 1.1% among patients treated with 1.8 mg/day of Victoza®. The clinical significance of these findings is unknown. Counsel patients regarding the risk for MTC and the symptoms of thyroid tumors (e.g. a mass in the neck, dysphagia, dyspnea or persistent hoarseness). It is unknown whether monitoring with serum calcitonin or thyroid ultrasound will mitigate the potential risk of MTC, and such monitoring may increase the risk of unnecessary procedures, due to low test specificity for serum calcitonin and a high background incidence of thyroid disease. Patients with thyroid nodules noted on physical examination or neck imaging obtained for other reasons should be referred to an endocrinologist for further evaluation. Although routine monitoring of serum calcitonin is of uncertain value in patients treated with Victoza®, if serum calcitonin is measured and found to be elevated, the patient should be referred to an endocrinologist for further evaluation. **Pancreatitis:** In clinical

trials of Victoza®, there were 7 cases of pancreatitis among Victoza®-treated patients and 1 case among comparator-treated patients (2.2 vs. 0.6 cases per 1000 patient-years). Five cases with Victoza® were reported as acute pancreatitis and two cases with Victoza® were reported as chronic pancreatitis. In one case in a Victoza®-treated patient, pancreatitis, with necrosis, was observed and led to death; however clinical causality could not be established. One additional case of pancreatitis has subsequently been reported in a Victoza®-treated patient. Some patients had other risk factors for pancreatitis, such as a history of cholelithiasis or alcohol abuse. There are no conclusive data establishing a risk of pancreatitis with Victoza® treatment. After initiation of Victoza®, and after dose increases, observe patients carefully for signs and symptoms of pancreatitis (including persistent severe abdominal pain, sometimes radiating to the back and which may or may not be accompanied by vomiting). If pancreatitis is suspected, Victoza® and other potentially suspect medications should be discontinued promptly, confirmatory tests should be performed and appropriate management should be initiated. If pancreatitis is confirmed, Victoza® should not be restarted. Use with caution in patients with a history of pancreatitis. **Use with Medications Known to Cause Hypoglycemia:** Patients receiving Victoza® in combination with an insulin secretagogue (e.g., sulfonylurea) may have an increased risk of hypoglycemia. In the clinical trials of at least 26 weeks duration, hypoglycemia requiring the assistance of another person for treatment occurred in 7 Victoza®-treated patients and in no comparator-treated patients. Six of these 7 patients treated with Victoza® were also taking a sulfonylurea. The risk of hypoglycemia may be lowered by a reduction in the dose of sulfonylurea or other insulin secretagogues [see *Adverse Reactions*]. **Macrovascular Outcomes:** There have been no clinical studies establishing conclusive evidence of macrovascular risk reduction with Victoza® or any other antidiabetic drug.

ADVERSE REACTIONS: Clinical Trials Experience: Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The safety of Victoza® was evaluated in a 52-week monotherapy trial and in four 26-week, add-on combination therapy trials. In the monotherapy trial, patients were treated with Victoza® 1.2 mg daily, Victoza® 1.8 mg daily, or glimepiride 8 mg daily. In the add-on to metformin trial, patients were treated with Victoza® 0.6 mg, Victoza® 1.2 mg, Victoza® 1.8 mg, placebo, or glimepiride 4 mg. In the add-on to glimepiride trial, patients were treated with Victoza® 0.6 mg, Victoza® 1.2 mg, Victoza® 1.8 mg, placebo, or rosiglitazone 4 mg. In the add-on to metformin + glimepiride trial, patients were treated with Victoza® 1.8 mg, placebo, or insulin glargine. In the add-on to metformin + rosiglitazone trial, patients were treated with Victoza® 1.2 mg, Victoza® 1.8 mg or placebo. **Withdrawals:** The incidence of withdrawal due to adverse events was 7.8% for Victoza®-treated patients and 3.4% for comparator-treated patients in the five controlled trials of 26 weeks duration or longer. This difference was driven by withdrawals due to gastrointestinal adverse reactions, which occurred in 5.0% of Victoza®-treated patients and 0.5% of comparator-treated patients. The most common adverse reactions leading to withdrawal for Victoza®-treated patients were nausea (2.8% versus 0% for comparator) and vomiting (1.5% versus 0.1% for comparator). Withdrawal due to gastrointestinal adverse events mainly occurred during the first 2-3 months of the trials. Tables 1 and 2 summarize the adverse events reported in ≥5% of Victoza®-treated patients in the five controlled trials of 26 weeks duration or longer.

Table 1: Adverse events reported in ≥ 5% of Victoza®-treated patients or ≥5% of glimepiride-treated patients: 52-week monotherapy trial

Adverse Event Term	All Victoza® N = 497 (%)	Glimepiride N = 248 (%)
Nausea	28.4	8.5
Diarrhea	17.1	8.9
Vomiting	10.9	3.6
Constipation	9.9	4.8
Upper Respiratory Tract Infection	9.5	5.6
Headache	9.1	9.3
Influenza	7.4	3.6
Urinary Tract Infection	6.0	4.0
Dizziness	5.8	5.2
Sinusitis	5.6	6.0
Nasopharyngitis	5.2	5.2
Back Pain	5.0	4.4
Hypertension	3.0	6.0

Table 2: Adverse events reported in ≥ 5% of Victoza®-treated patients and occurring more frequently with Victoza® compared to placebo: 26-week combination therapy trials

Add-on to Metformin Trial			
	All Victoza® + Metformin N = 724	Placebo + Metformin N = 121	Glimepiride + Metformin N = 242
Adverse Event Term	(%)	(%)	(%)
Nausea	15.2	4.1	3.3
Diarrhea	10.9	4.1	3.7
Headache	9.0	6.6	9.5
Vomiting	6.5	0.8	0.4
Add-on to Glimepiride Trial			
	All Victoza® + Glimepiride N = 695	Placebo + Glimepiride N = 114	Rosiglitazone + Glimepiride N = 231
Adverse Event Term	(%)	(%)	(%)
Nausea	7.5	1.8	2.6
Diarrhea	7.2	1.8	2.2
Constipation	5.3	0.9	1.7
Dyspepsia	5.2	0.9	2.6

Add-on to Metformin + Glimepiride			
	Victoza® 1.8 + Metformin + Glimepiride N=230	Placebo + Metformin + Glimepiride N=114	Glargine + Metformin + Glimepiride N=232
Adverse Event Term	(%)	(%)	(%)
Nausea	13.9	3.5	1.3
Diarrhea	10.0	5.3	1.3
Headache	9.6	7.9	5.6
Dyspepsia	6.5	0.9	1.7
Vomiting	6.5	3.5	0.4
Add-on to Metformin + Rosiglitazone			
	All Victoza® + Metformin + Rosiglitazone N = 355	Placebo + Metformin + Rosiglitazone N = 175	
Adverse Event Term	(%)	(%)	
Nausea	34.6	8.6	
Diarrhea	14.1	6.3	
Vomiting	12.4	2.9	
Decreased Appetite	9.3	1.1	
Anorexia	9.0	0.0	
Headache	8.2	4.6	
Constipation	5.1	1.1	
Fatigue	5.1	1.7	

Gastrointestinal adverse events: In the five clinical trials of 26 weeks duration or longer, gastrointestinal adverse events were reported in 41% of Victoza®-treated patients and were dose-related. Gastrointestinal adverse events occurred in 17% of comparator-treated patients. Events that occurred more commonly among Victoza®-treated patients included nausea, vomiting, diarrhea, dyspepsia and constipation. In clinical trials of 26 weeks duration or longer, the percentage of patients who reported nausea declined over time. Approximately 13% of Victoza®-treated patients and 2% of comparator-treated patients reported nausea during the first 2 weeks of treatment. **Immunogenicity:** Consistent with the potentially immunogenic properties of protein and peptide pharmaceuticals, patients treated with Victoza® may develop anti-liraglutide antibodies. Approximately 50-70% of Victoza®-treated patients in the five clinical trials of 26 weeks duration or longer were tested for the presence of anti-liraglutide antibodies at the end of treatment. Low titers (concentrations not requiring dilution of serum) of anti-liraglutide antibodies were detected in 8.6% of these Victoza®-treated patients. Sampling was not performed uniformly across all patients in the clinical trials, and this may have resulted in an underestimate of the actual percentage of patients who developed antibodies. Cross-reacting anti-liraglutide antibodies to native glucagon-like peptide-1 (GLP-1) occurred in 6.9% of the Victoza®-treated patients in the 52-week monotherapy trial and in 4.8% of the Victoza®-treated patients in the 26-week add-on combination therapy trials. These cross-reacting antibodies were not tested for neutralizing effect against native GLP-1, and thus the potential for clinically significant neutralization of native GLP-1 was not assessed. Antibodies that had a neutralizing effect on liraglutide in an *in vitro* assay occurred in 2.3% of the Victoza®-treated patients in the 52-week monotherapy trial and in 1.0% of the Victoza®-treated patients in the 26-week add-on combination therapy trials. Among Victoza®-treated patients who developed anti-liraglutide antibodies, the most common category of adverse events was that of infections, which occurred among 40% of these patients compared to 36%, 34% and 35% of antibody-negative Victoza®-treated, placebo-treated and active-control-treated patients, respectively. The specific infections which occurred with greater frequency among Victoza®-treated antibody-positive patients were primarily nonserious upper respiratory tract infections, which occurred among 11% of Victoza®-treated antibody-positive patients; and among 7%, 7% and 5% of antibody-negative Victoza®-treated, placebo-treated and active-control-treated patients, respectively. Among Victoza®-treated antibody-negative patients, the most common category of adverse events was that of gastrointestinal events, which occurred in 43%, 18% and 19% of antibody-negative Victoza®-treated, placebo-treated and active-control-treated patients, respectively. Antibody formation was not associated with reduced efficacy of Victoza® when comparing mean HbA_{1c} of all antibody-positive and all antibody-negative patients. However, the 3 patients with the highest titers of anti-liraglutide antibodies had no reduction in HbA_{1c} with Victoza® treatment. In clinical trials of Victoza®, events from a composite of adverse events potentially related to immunogenicity (e.g., urticaria, angioedema) occurred among 0.8% of Victoza®-treated patients and among 0.4% of comparator-treated patients. Urticaria accounted for approximately one-half of the events in this composite for Victoza®-treated patients. Patients who developed anti-liraglutide antibodies were not more likely to develop events from the immunogenicity events composite than were patients who did not develop anti-liraglutide antibodies. **Injection site reactions:** Injection site reactions (e.g., injection site rash, erythema) were reported in approximately 2% of Victoza®-treated patients in the five clinical trials of at least 26 weeks duration. Less than 0.2% of Victoza®-treated patients discontinued due to injection site reactions. **Papillary thyroid carcinoma:** In clinical trials of Victoza®, there were 6 reported cases of papillary thyroid carcinoma in patients treated with Victoza® and 1 case in a comparator-treated patient (1.9 vs. 0.6 cases per 1000 patient-years). Most of these papillary thyroid carcinomas were <1 cm in greatest diameter and were diagnosed in surgical pathology specimens after thyroidectomy prompted by findings on protocol-specified screening with serum calcitonin or thyroid ultrasound. **Hypoglycemia:** In the clinical trials of at least 26 weeks duration, hypoglycemia requiring the assistance of another person for treatment occurred in 7 Victoza®-treated patients (2.6 cases per 1000 patient-years) and in no comparator-treated patients. Six of these 7 patients treated

with Victoza® were also taking a sulfonylurea. One other patient was taking Victoza® in combination with metformin but had another likely explanation for the hypoglycemia (this event occurred during hospitalization and after insulin infusion) (Table 3). Two additional cases of hypoglycemia requiring the assistance of another person for treatment have subsequently been reported in patients who were not taking a concomitant sulfonylurea. Both patients were receiving Victoza®, one as monotherapy and the other in combination with metformin. Both patients had another likely explanation for the hypoglycemia (one received insulin during a frequently-sampled intravenous glucose tolerance test, and the other had intracranial hemorrhage and uncertain food intake).

Table 3: Incidence (%) and Rate (episodes/patient year) of Hypoglycemia in the 52-Week Monotherapy Trial and in the 26-Week Combination Therapy Trials

	Victoza® Treatment (N = 497)	Active Comparator Glimepiride (N = 248)	Placebo Comparator None
Monotherapy			
Patient not able to self-treat	0	0	—
Patient able to self-treat	9.7 (0.24)	25.0 (1.66)	—
Not classified	1.2 (0.03)	2.4 (0.04)	—
Add-on to Metformin	Victoza® + Metformin (N = 724)	Glimepiride + Metformin (N = 242)	Placebo + Metformin (N = 121)
Patient not able to self-treat	0.1 (0.001)	0	0
Patient able to self-treat	3.6 (0.05)	22.3 (0.87)	2.5 (0.06)
Add-on to Glimepiride	Victoza® + Glimepiride (N = 695)	Rosiglitazone + Glimepiride (N = 231)	Placebo + Glimepiride (N = 114)
Patient not able to self-treat	0.1 (0.003)	0	0
Patient able to self-treat	7.5 (0.38)	4.3 (0.12)	2.6 (0.17)
Not classified	0.9 (0.05)	0.9 (0.02)	0
Add-on to Metformin + Rosiglitazone	Victoza® + Metformin + Rosiglitazone (N = 355)	None	Placebo + Metformin + Rosiglitazone (N = 175)
Patient not able to self-treat	0	—	0
Patient able to self-treat	7.9 (0.49)	—	4.6 (0.15)
Not classified	0.6 (0.01)	—	1.1 (0.03)
Add-on to Metformin + Glimepiride	Victoza® + Metformin + Glimepiride (N = 230)	Insulin glargine + Metformin + Glimepiride (N = 232)	Placebo + Metformin + Glimepiride (N = 114)
Patient not able to self-treat	2.2 (0.06)	0	0
Patient able to self-treat	27.4 (1.16)	28.9 (1.29)	16.7 (0.95)
Not classified	0	1.7 (0.04)	0

In a pooled analysis of clinical trials, the incidence rate (per 1,000 patient-years) for malignant neoplasms (based on investigator-reported events, medical history, pathology reports, and surgical reports from both blinded and open-label study periods) was 10.9 for Victoza®, 6.3 for placebo, and 7.2 for active comparator. After excluding papillary thyroid carcinoma events [see Adverse Reactions], no particular cancer cell type predominated. Seven malignant neoplasm events were reported beyond 1 year of exposure to study medication, six events among Victoza®-treated patients (4 colon, 1 prostate and 1 nasopharyngeal), no events with placebo and one event with active comparator (colon). Causality has not been established. **Laboratory Tests:** In the five clinical trials of at least 26 weeks duration, mildly elevated serum bilirubin concentrations (elevations to no more than twice the upper limit of the reference range) occurred in 4.0% of Victoza®-treated patients, 2.1% of placebo-treated patients and 3.5% of active-comparator-treated patients. This finding was not accompanied by abnormalities in other liver tests. The significance of this isolated finding is unknown.

OVERDOSAGE: In a clinical trial, one patient with type 2 diabetes experienced a single overdose of Victoza® 17.4 mg subcutaneous (10 times the maximum recommended dose). Effects of the overdose included severe nausea and vomiting requiring hospitalization. No hypoglycemia was reported. The patient recovered without complications. In the event of overdose, appropriate supportive treatment should be initiated according to the patient's clinical signs and symptoms.

More detailed information is available on request.

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Victoza® is a registered trademark of Novo Nordisk A/S. Victoza® is covered by US Patent Nos. 6,268,343; 6,458,924; and 7,235,627 and other patents pending. Victoza® Pen is covered by US Patent Nos. 6,004,297; 6,235,004; 6,582,404 and other patents pending.

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