Archives of Physiology and Biochemistry, 2013; 119(1): 1–14

ISSN 1381-3455 print/ISSN 1744-4160 online
DOI: 10.3109/13813455.2012.754474

informa
healthcare

ORIGINAL ARTICLE

The metabolic and mitogenic properties of basal insulin analogues

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Abstract

Context: Retrospective, observational studies have reported an association between diabetes treatment with insulin and a higher incidence of cancer.

Objective: Overview the literature for *in vitro* and *in vivo* studies of the metabolic and mitogenic properties of basal insulin analogues and assess the implications for clinical use.

Methods: Relevant studies were identified through PubMed and congress abstract database searches; data on metabolic and mitogenic signalling in relation to insulin treatment of diabetes are included in this review.

Results: The balance of evidence shows that although some analogues have demonstrated mitogenic potency in some *in vitro* studies in cancer cell lines, these findings do not translate to the *in vivo* setting in animals or to the clinical setting in humans.

Conclusions: The current consensus is that there is no clinical or *in vivo* evidence to indicate that any commercially available insulin analogue has carcinogenic effects. Large-scale, prospective clinical and observational studies will further establish any potential link.

Keywords: Hyperinsulinaemia, IGF-1, insulin receptor

Introduction

There is growing evidence of an association between diabetes and the incidence of cancer (Giovannucci *et al.*, 2010). Several studies have identified an increased risk of cancer in patients with both type 1 and type 2 diabetes (Saydah *et al.*, 2003; Coughlin *et al.*, 2004; Smith & Gale, 2009; Jalving *et al.*, 2010). The reason behind this association is the subject of much debate and a number of factors could potentially play a role. The link between diabetes and cancer may be indirect and associated with risk factors common to both conditions, such as obesity. Alternatively, there may be a direct causal link due to metabolic disturbances, such as hyperglycaemia, insulin resistance or hyperinsulinaemia, and there are also suggestions that diabetes therapy may be a factor. It is recognized that insulin has dose-related effects on cell proliferation and differentiation (Sandow, 2009) and recent epidemiological studies have also suggested an association between some insulin analogues and an increased risk of developing cancer (Bowker *et al.*, 2006; Currie *et al.*, 2009; Hemkens *et al.*, 2009; Jonasson *et al.*, 2009; Colhoun, 2009; Chang *et al.*, 2011; Morden *et al.*, 2011; Ruiter *et al.*, 2011).

However, much of the evidence for the association between cancer and diabetes treatment comes from retrospective studies with confounding factors that limit interpretation. In principle, retrospective studies are unable to determine a cause-and-effect relationship. One method of progressing the question of a link between diabetes, its treatment and cancer would be to prospectively assess these questions, but there is a dearth of such data at present (Bowker *et al.*, 2006; Farooki & Schneider, 2006; Smith & Gale, 2009). With regard to therapy, it is also important to understand any underlying pathophysiological mechanisms that involve insulin, insulin analogues and cancer. In response, here

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(Received 19 October 2012; revised 21 November 2012; accepted 26 November 2012)

we review the current literature on pathophysiological mechanisms, including metabolic and mitogenic effects of three long-acting basal insulin analogues, with the aim of assessing the potential link between long-acting insulin analogues and the incidence of cancer.

Methods

Search criteria and analyses

Appropriate studies for inclusion in this review were identified by searching the PubMed database using various combinations of the following terms: cancer, carcinogenic, carcinogenicity, malignancy, malignancies, mitogenic, mitogenicity, metabolic, Type 1 diabetes, Type 2 diabetes, basal insulin, insulin analogues, insulin glargine, insulin detemir, insulin degludec. Congress abstract databases such as the American Diabetes Association and European Association for the Study of Diabetes were also searched. No restrictions on publication date were enforced in order to capture all relevant analyses. Consideration was also given to references detailed in individual publications which did not feature in the original search results. The authors reviewed the resultant studies; data on metabolic and mitogenic signalling in relation to insulin treatment of diabetes are included in this review.

Insulin, insulin-like growth factor-1 and their receptors

The theoretical basis for a biological link between insulin analogues and cancer relates to possible differences in stimulation of insulin and insulin-like growth factor (IGF) receptors by insulin analogues compared with endogenous insulin. The ubiquitous insulin and IGF receptors belong to the receptor tyrosine kinase superfamily and share substantial structural homology. Both insulin receptors (IRs) and IGF-1 receptors (IGF-1Rs) are dimers consisting of two extracellular ligand-binding alpha subunits and two transmembrane beta subunits containing the tyrosine kinase domain. Ligand activation results in transphosphorylation of the kinase domains that triggers shared intracellular signalling pathways for metabolic and mitogenic processes (De Meyts & Wittaker, 2002; De Meyts *et al.*, 2004).

Overview of receptor types

The IRs and IGF-1Rs share more than 50% of their amino acid sequence and have more than 80% homology in their β-subunit tyrosine kinase domains (Ullrich *et al.*, 1986; Chisalita & Arnqvist, 2004). The second highest degree of sequence homology (64–67%) arises in the extracellular α-subunit regions flanking the cysteine-rich subdomains, although similarity is lower within the α-subunit cysteine-rich domain (48%) (Figure 1) (Ullrich *et al.*, 1986). There are two isoforms of human IR, IR-A (short form) and IR-B (long form), which differ from each other by 12 amino acids and are variably expressed in different tissues (Frasca *et al.*, 1999), with IR-A found predominantly in central nervous system and haematopoietic cells and IR-B found predominantly in adipose tissue, liver and muscle (Moller *et al.*, 1989; Mosthaf *et al.*, 1990). IR-A binds both insulin and IGF-2, a growth factor protein similar to IGF-1, which shares homology with insulin (Frasca *et al.*, 1999; Belfiore *et al.*, 2009). The affinity of insulin for IR is approximately 100- to 1000-fold greater than for IGF-1R and the same is true for the affinity of IGF-1 for IGF-1R compared with IR; therefore, at physiological concentrations, little receptor cross-talk occurs (Chisalita & Arnqvist, 2004). Insulin and IGF-1 half-receptors can also heterodimerize to form insulin/IGF-1 hybrid receptors that bind IGF-1 with high affinity (Pandini *et al.*, 2002).

Receptor expression patterns and function

Most mammalian cells express both IRs and IGF-1Rs. Of the two IR isoforms, IR-A is predominantly expressed during embryogenesis and prenatal development, enhancing the effects of IGF-2, which has been shown to play a role in embryonic development and carcinogenesis (Frasca *et al.*, 1999). However, IR-A expression can also be detected in adult tissues, including the brain, but at a lower degree than IR-B. In contrast, IR-B is predominantly expressed in adult, well-differentiated cells (Belfiore *et al.*, 2009). The IR is mainly involved in mediating metabolic intracellular signalling cascades (protein kinase B (PKB) signalling pathways), whereas the IGF-1R primarily initiates growth and differentiation activities (mitogen-activated protein (MAP) kinase signalling pathways) (LeRoith *et al.*, 1995; Taniguchi *et al.*, 2006). Both the insulin and IGF receptors trigger a complex variety of intracellular signals for metabolism, cell growth and proliferation (Taniguchi *et al.*, 2006). The relative abundance of the IR isoforms affects the intracellular signalling activated by insulin/IGF-1 hybrid receptors, which has important consequences for tissue-specific responses to insulin, IGFs and insulin analogues (Pandini *et al.*, 2002).

Receptor function in cancer cells

Malignant cell growth is often associated with aberrant signalling of both IR and IGF-1R. Overexpression of IR and IGF-1R often coincides with human breast carcinomas, which allows insulin/IGF-1 hybrid receptors to form. These hybrid receptors become tyrosine autophosphorylated when breast cancer cells are exposed to IGF-1 but not insulin and, furthermore, the hybrid receptors mediate growth in response to IGF-1 (Moxham *et al.*, 1989; Soos *et al.*, 1993; Pandini *et al.*, 1999; Belfiore *et al.*, 2009).

IR-A may also play a key role in the development of cancer when activated by IGF-2 in breast cancer cell lines (Sciacca *et al.*, 1999) and thyroid cancer (Vella *et al.*, 2002) *in vitro*. IR-A activation by IGF-2 leads to the recruitment of different intracellular signalling proteins compared with IR-A activation by human insulin (Frasca *et al.*, 1999). Frasca *et al.* (1999) showed that when IR-A is activated by insulin, the effects are mainly metabolic, whereas activation by IGF-2 leads to mitogenic effects.

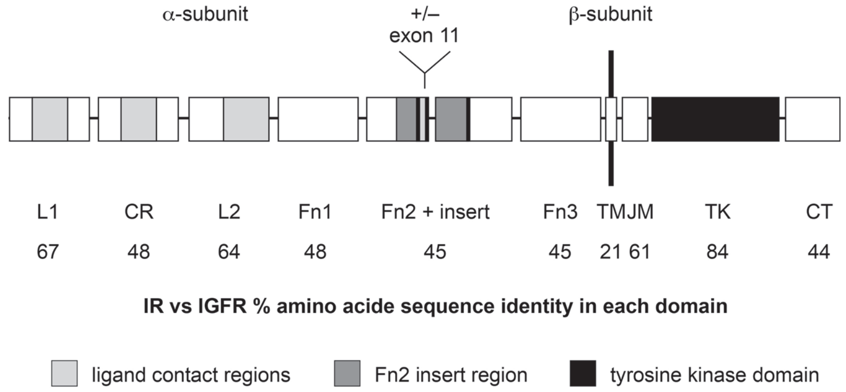

Figure 1. Schematic illustration of the sequence homology between the IR and IGF-R (Siddle *et al.*, 2001). IR = insulin receptor; IGF-R = insulin growth factor receptor. Copyright requested.

The interaction between IR-A and IGF-2 may play a role in both foetal growth and cancer development. IR-A expression is also often aberrant in cancer cells, increasing their responsiveness to IGF-2, which stimulates cell growth; IR-A overexpression also sensitizes cancer cells to circulating insulin, which may explain the cancer-promoting effect of hyperinsulinaemia observed in both obese individuals and those with type 2 diabetes (Belfiore *et al.*, 2009).

In addition, IGF-1R has been implicated in breast cancer development, including proliferation, survival, transformation, differentiation, cell–cell and cell–substrate interactions (Surmacz, 2000). *In vitro* experiments show that oestrogen receptor-positive cells respond to activation of the IGF-1R/insulin receptor substrate-1 pathway by improving growth and counteracting apoptosis induced by anticancer treatments, whereas breast cancer cells with no or low levels of the oestrogen receptor often express low levels of IGF-1R and fail to respond to IGF-1 with mitogenesis (Bartucci *et al.*, 2001; Bhargava *et al.*, 2011).

As cancer cells have aberrant IR and IGF-1R signalling patterns, it is important to understand how insulin analogues affect both normal and cancerous cells, as this will have implications for diabetes, cancer and cancer treatment. For example, an early investigational insulin analogue, Asp^{B10} insulin, was shown to have not only increased binding affinity for IR, but also an increased occupancy time, which led to prolonged signalling, increasing its metabolic and mitogenic potency (Berti *et al.*, 1998; Kurtzhals *et al.*, 2000).

Long-acting insulin analogues and their metabolites

Insulin analogues contain modifications to the insulin structure, primarily to improve their pharmacokinetic profiles compared with human insulin and thus overcome the limitations of traditional insulin preparations (such as the intermediate-acting neutral protamine Hagedorn [NPH] insulin; Novo Nordisk). Conventional human insulin often results in wide glucose fluctuations and adverse effects such as hypoglycaemia, preventing treatment targets being achieved (1998). Insulin analogues are advantageous compared with human insulin as they have less pharmacokinetic variability and profiles that are better adapted to their specific requirements, either rapid- or long-acting. This translates into improved safety and efficacy (Guerci & Sauvanet, 2005). In particular, insulin analogues are more likely to mimic the physiological pattern of endogenous insulin secretion, reduce the risk of hypoglycaemia and provide greater flexibility for patients compared with human insulin, encouraging better glycaemic control (Bell, 2007). Currently approved long-acting basal insulin analogues are insulin glargine (Lantus®; Sanofi) and insulin detemir (Levemir®, Novo Nordisk) (Figure 2). Insulin degludec (Novo Nordisk), another long-acting insulin analogue, is currently in late-stage clinical development (Figure 2).

Given that different insulin analogues have different amino acid residue deletions, substitutions and additions, one can assume that their affinity to IR and IGF-1R may also vary. For example, modifications to amino acid B10 are able to increase the affinity and potency of insulin analogues for both the IR and IGF-1R, while addition of residues at B31 or B32 may enhance the binding affinity of an insulin analogue to IGF-1R but not IR, and deletion of B26–B30 residues decreases binding affinity of the insulin analogue to the IGF-1R, but only moderately to the IR (Slieker *et al.*, 1997).

Insulin glargine was the first long-acting insulin analogue to be approved. It differs from human insulin by the substitution of asparagine with glycine at position 21 on the A-chain and the addition of two arginine residues at the carboxy-terminal end of the B-chain (Rosskamp & Park, 1999). The addition of the arginine residues causes a shift in the isoelectric point of the molecule making it less soluble at a neutral pH. As a result, after *in vivo* subcutaneous injection, glargine precipitates into multi-hexamers in subcutaneous tissue forming a local depot with a slow dissolution into dimers and then monomers (which exert the biological activity of the analogue), thereby extending its duration of action (Berchtold & Hilgenfeld, 1999). The slow release of insulin glargine into solution is accompanied by biotransformation as it undergoes rapid cleavage at the carboxy-terminus of

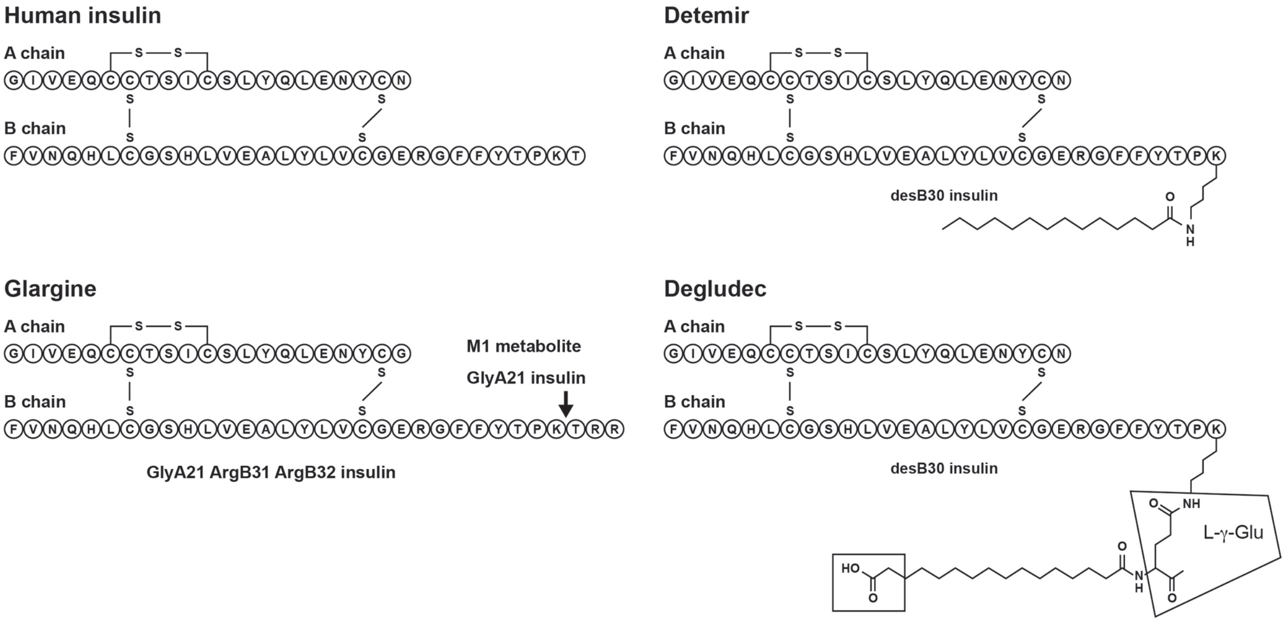

Figure 2. Schematic diagram showing the modifications to the insulin structure for the long-acting insulin analogues, insulins glargine, detemir and degludec (Agin *et al.*, 2007). Copyright requested.

the B-chain forming the active metabolite M1 ([Gly^{A21}] insulin), which lacks the di-arginine residues (Figure 3) (Kuerzel *et al.*, 2003). The threonine at position 30 on the B-chain is also subsequently cleaved to form a second active metabolite M2 ([Gly^{A21}, des-Thr^{B30}] insulin) (Kuerzel *et al.*, 2003). It has been demonstrated consistently *in vivo* across a range of animals (rats, dogs, diabetic pigs), and in individuals with either type 1 or type 2 diabetes, that almost all administered insulin glargine is rapidly converted into the M1 metabolite (Bolli *et al.*, 2011; Lucidi *et al.*, 2011; Werner *et al.*, 2012), accounting for most (~90%) of the daily plasma insulin (Lucidi *et al.*, 2011). This has been observed with both subcutaneous and intravenous administration of insulin glargine (Werner *et al.*, 2012). Since it is the predominant metabolite in all species investigated, M1 – as opposed to the insulin glargine molecule itself – is the primary driver of the pharmacodynamic effect and the long-acting time–action profile observed with insulin glargine treatment (Bolli *et al.*, 2011; Lucidi *et al.*, 2011; Werner *et al.*, 2012). The metabolism of insulin glargine is initiated at the site of injection and then continues in the circulatory system (Ciaraldi & Sasaoka, 2011).

Albumin-bound drugs

Insulin detemir is an acylated insulin analogue with the lysine at position 29 on the B-chain modified with a C14 myristic fatty acid group, allowing it to reversibly bind to human serum albumin (HSA) (Olsen & Kaarsholm, 2000). The rate of metabolism of insulin detemir is similar to human insulin with terminal half-lives of 5–7 hours and 4–6 hours, respectively, and all metabolites are inactive (Duckworth *et al.*, 1998; Levemir® Prescribing Information, 2009). After subcutaneous injection, 98% of insulin detemir binds to HSA via its fatty acid side chain, thereby prolonging its duration of action by providing a 'floating depot' but also reducing its biological availability (Markussen *et al.*, 1996; Wada *et al.*, 2008; Vigneri *et al.*, 2010). Insulin detemir must be administered at 0.142 mg of the analogue unit instead of 0.036 mg per unit for human insulin or other insulin analogues (3.9-fold higher insulin molecule number per unit) (Vigneri *et al.*, 2010). This four-fold lower potency of insulin detemir relative to that of human insulin is due to the myristic acid moiety being sufficiently close to the receptor recognition site so as to interfere with insulin receptor binding (Levemir: EPAR European Medical Association, 2004). Achieving maximal glucose uptake response with insulin detemir has been shown to require a 10-fold higher plasma insulin concentration than with insulin glargine or human insulin (Wada *et al.*, 2008).

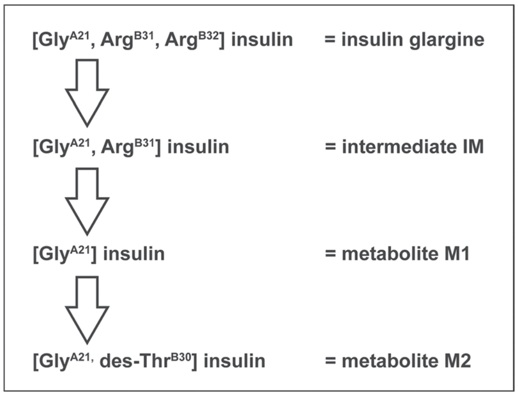

Figure 3. Schematic diagram showing the metabolism of insulin glargine (Sommerfeld *et al.*, 2010).

Insulin degludec is an insulin analogue with deletion of Thr^{B30} and the addition of a 16-carbon fatty di-acid attached to Lys^{B29} via a glutamic acid spacer. It has been proposed that its half-life of ~25 hours in humans results from the dissolution of multi-hexamer assemblies after subcutaneous injection (Jonassen *et al.*, 2010; 2012). However, data from Jonassen *et al.* (2012) suggest that, similar to insulin detemir, the molecular design of insulin degludec also results in HSA binding, albeit with a lower affinity (insulin detemir = 1.0, insulin degludec = 2.4). Binding to HSA by insulin degludec is reflected in higher circulating insulin levels compared with human insulin and other insulin analogues as well as reduced bioactivity of *in vitro* assays.

Although there are no published studies regarding the metabolism of insulin degludec to date, due to their structural similarities (Figure 2), insulin detemir and insulin degludec may be metabolized in a similar manner. Insulin detemir is initially cleaved at the disulphide bridges after which the A- and B-chains are hydrolysed to smaller inactive metabolites that incorporate the lysine residue at B29 and all or part of the myristic acid moiety attached to its ε-amino group, which are further degraded (Levemir: EPAR European Medical Association, 2004). Once more information on insulin degludec is published; it will be interesting to see if its structural similarities with insulin detemir confer other similar traits, such as HSA binding affinity or a potential requirement for a higher concentration at higher BMIs.

In vitro studies of long-acting insulin analogues

Receptor-binding characteristics and the potential for IGF-1-like activity of the insulin analogues have been studied *in vitro* using various techniques, including cell lines, primary cell cultures and solubilized receptors. The majority of studies have investigated the IR and IGF-1R binding properties of insulin glargine, but there is a dearth of comparative data with insulins detemir and degludec.

Insulin receptor-binding characteristics of long-acting insulin analogues

In a study by Kurtzhals *et al.* (2000), insulin glargine was shown to have 86% binding affinity for the IR with an off-rate of 152% (1.5-fold accelerated dissociation) compared with human insulin. In a more recent *in vitro* study, however, insulin glargine showed about 50% less binding affinity for the two IR isoforms than human insulin, which correlated well with the 40–50% lower metabolic activity (Table 1) (Sommerfeld *et al.*, 2010). The rapid metabolism of insulin glargine into its active metabolites M1 and M2 makes the binding properties of these metabolites of key interest. The M1 insulin glargine metabolite had a similar affinity as insulin glargine for the IR (78% relative to human insulin), with an off-rate of 162% (Kurtzhals *et al.*, 2000). Insulin detemir had a relative affinity for the IR of 46% and an off-rate of 204%; the reduced affinity may result from the C14 fatty acid attached to Lys^{B29} making hydrophobic contacts with one or more of the aromatic residues in positions B24–B25, thereby shielding these residues from recognition by the IR (Kurtzhals *et al.*, 2000). So far, there has only been one report for insulin degludec's affinity for the IR, which has a relative affinity of 13–15% compared with human insulin (Nishimura *et al.*, 2010). The dissociation of insulin degludec from IR has not yet been reported.

Structure–function studies have suggested that insulin analogues with a reduced rate of dissociation from the insulin receptor are linked to higher mitogenic potency than metabolic potency versus human insulin (Hansen *et al.*, 1996; Kurtzhals *et al.*, 2000). AspB10, for example, the only analogue with known carcinogenicity, has an off-rate of 14% (Kurtzhals *et al.*, 2000). As described above, insulins glargine and detemir have faster dissociation rates from the insulin receptor than human insulin, suggesting a lower mitogenic potency than human insulin.

Insulin receptor phosphorylation and intracellular signalling stimulated by long-acting insulin analogues

Evidence suggests that insulin analogues have different effects on IR phosphorylation that result in different intracellular signalling properties in various cell types. In fact, consideration of the complexity of the AKT and ERK signalling pathways, primarily responsible for metabolic

Table 1. Summarized *in vitro* data for insulin, IGFs and glargine metabolites (Sommerfeld et al., 2010; Yehezkel et al., 2010).

Analogue	IR-A affinity IC_{50} (nmol/L)	IR-B affinity IC_{50} (nmol/L)	IR-A autophosphorylation EC_{50} (nmol/L)	IR-B autophosphorylation EC_{50} (nmol/L)	Metabolic potency EC_{50} (nmol/L)	IGF-1R affinity IC_{50} (nmol/L)	IGF-1R autophosphorylation EC_{50} (nmol/L)	Mitogenic potency EC_{50} (nmol/L)
Human insulin	0.49 ± 0.04	0.57 ± 0.02	11.0 ± 1.3	11.7 ± 1.4	0.045 ± 0.003	289 ± 53.3	447 ± 38.7	12.25 ± 0.27
Glargine	0.83 ± 0.08	1.10 ± 0.12	18.4 ± 2.4	23.6 ± 2.1	0.066 ± 0.005	63.2 ± 19.9	87.5 ± 10	1.61 ± 0.26
Glargine IM	0.78 ± 0.03	1.08 ± 0.06	23.1 ± 2.7	20.9 ± 1.3	0.098 ± 0.012	80.0 ± 10.2	179 ± 19.6	3.75 ± 0.31
Glargine M1	1.02 ± 0.03	1.35 ± 0.20	18.6 ± 2.5	18.1 ± 1.7	0.139 ± 0.009	649 ± 31.9	644 ± 56.9	16.25 ± 2.35
Glargine M2	0.93 ± 0.16	1.09 ± 0.06	19.2 ± 1.6	21.4 ± 2.3	0.087 ± 0.007	427 ± 20.6	485 ± 43.6	17.90 ± 6.50
$[\text{Asp}^{B10}]$ insulin	0.06 ± 0.01	0.21 ± 0.03	5.1 ± 0.7	3.32 ± 0.78	0.031 ± 0.005	104 ± 12.8	72.7 ± 7.6	1.52 ± 0.15
IGF-1	64.5 ± 5.1	171 ± 50	449 ± 61.7	>1000	19.01 ± 0.93	0.89 ± 0.19	2.9 ± 0.4	0.22 ± 0.05
IGF-2	6.2 ± 0.34	46.6 ± 7.8	85.4 ± 5.5	384 ± 68.4	–	6.68 ± 2.24	–	–

Data are means ± standard error of the mean. All analogues were tested at least three times on different days. Activity was determined within each experiment and then averaged to yield a single reported mean. IR = insulin receptor; IGF-1R = insulin-like growth factor 1 receptor; IM = intermediate ($[\text{Gly}^{A21}, \text{Arg}^{B31}]$ insulin); M1 = metabolite 1 ($[\text{Gly}^{A21}]$ insulin); M2 = metabolite 2 ($[\text{Gly}^{A21}$,des-$\text{Thr}^{B30}]$ insulin); IGF = insulin-like growth factor. Table reproduced from Sommerfeld *et al.*, 2010. *In vitro* metabolic and mitogenic signaling of insulin glargine and its metabolites. *PLoS One* 5: e9540.

and mitogenic activities, respectively, helps to explain why even minor conformational differences induced in the receptor molecule by different ligands can alter the signalling cascade (Vigneri *et al.*, 2010). A study by Sciacca *et al.* (2010) in three engineered cell models (mouse embryonic fibroblasts with a target disruption of the IGF-1R gene [R-] with either human IR-A, IR-B or IGF-1R) reported that long-acting insulin analogues had a phosphorylation pattern of both IR-A and IR-B that was similar to native insulin. However, in contrast, significant differences between native insulin and insulins glargine and detemir were observed in intracellular signalling properties, with an ultimate ERK/AKT activation ratio in favour of ERK for both IR isoforms with the insulin analogues (Sciacca *et al.*, 2010). However, at the concentrations tested (5 nmol/L for insulin glargine and 19 nmol/L for insulin detemir), neither insulin analogue demonstrated increased transforming activity.

In cancer cells, Weinstein *et al.* (2009) showed that both insulins glargine and detemir at a concentration of 50 ng/mL were able to phosphorylate IR in HCT-116 colorectal cancer cells, while in another study using HCT-116 cells, maximal IR phosphorylation was observed with insulin glargine at 25 ng/mL (Yehezkel *et al.*, 2010). However, in both studies, supraphysiological concentrations of insulin analogues were used that would translate to plasma concentrations that could never be achieved in patients with diabetes. This makes it difficult to determine the clinical relevance of such experiments; besides the fact these insulin analogues are able to activate the IR.

The same holds true for *in vitro* intracellular signalling experiments. Wada *et al*, showed that insulin detemir has reduced phosphorylatory effects on intracellular proteins and different signalling effects in various cell types compared with human insulin and insulin glargine (Wada *et al.*, 2008); these differences may be related to differences in the expression of IGF-1R and IR isoforms between the cell lines investigated (Serrano *et al.*, 2005; Sommerfeld *et al.*, 2010). Insulin detemir was less potent than insulin glargine or human insulin with respect to IR phosphorylation, which resulted in a weaker signal transduction via phosphatidylinosital-3-kinase and reduced glucose uptake. Human insulin and insulin glargine (both at the supraphysiological concentration of 50 ng/mL) have been shown to stimulate the phosphorylation of AKT (metabolic signalling pathway) in HCT-116 cells, whereas insulin detemir had a small phosphorylatory effect that was similar to that of IGF-1 (Weinstein *et al.*, 2009; Yehezkel *et al.*, 2010). It is likely that the difference in the amount of phosphorylated intracellular signal cascade proteins produced by human insulin and insulin glargine relative to insulin detemir are not the result of differential kinetic processes but the result of the steric hindrance, caused by the C14 fatty acid attached to the Lys^{B29}, in binding to the IR recognition site, resulting in a weaker transduction signal (Kurtzhals *et al.*, 2000).

There is currently no published data regarding receptor activation and intracellular signalling using insulin degludec.

Metabolic properties of long-acting insulin analogues

The metabolic activity of insulin and its analogues is commonly assessed *in vitro* by studies using glucose uptake, stimulation of lipogenesis and/or inhibition of lipolysis (Sandow, 2009). As already stated, once injected, insulin glargine is rapidly converted into its pharmacologically active metabolites; therefore, *in vivo* exposure to insulin glargine is likely to be minimal (Lucidi *et al.*, 2011; Bolli *et al.*, 2011; Werner *et al.*, 2012). The main metabolite, M1, accounts for almost all of the pharmacodynamic effects of subcutaneously injected insulin glargine (Lucidi *et al.*, 2011; Bolli *et al.*, 2011; Werner *et al.*, 2012). M1 had similar metabolic potential compared with human insulin as assessed in isolated primary mouse adipocytes (Kurtzhals *et al.*, 2000). In these cells, human insulin was found to stimulate lipogenesis approximately 12-fold over the basal level; insulin glargine, M1 and insulin detemir had metabolic potencies of 60, 88 and ~27% relative to human insulin, respectively. The measurement for detemir was estimated as it was assumed that only free insulin detemir is biologically active and it was calculated that 93.7% was albumin bound (Kurtzhals *et al.*, 2000). Similar assessments of lipogenesis by Sommerfeld *et al.* (2010) in primary rat adipocytes support these findings. Asp^{B10} insulin and IGF-1 showed the highest and lowest metabolic activities, respectively (Table 1). The metabolic activity of insulin glargine and its M1 metabolite was lower than that of human insulin as shown by half maximal effective concentration (EC$_{50}$) values that were 1.4- and 3.0-fold higher, respectively. The metabolic activity of two other insulin glargine metabolites, M2 and IM, were reported for the first time in this study and shown to be less active than human insulin and glargine, but slightly more active than M1 (Table 1).

With respect to glucose uptake, a recent study testing an assay measuring glucose transporter type 4 (GLUT4) translocation in rat myoblasts has shown that insulin glargine behaves much like insulin and not like IGF-1 (Baus *et al.*, 2010). Following an investigation of radioactive glucose uptake, IGF-1 was reported to have an EC$_{50}$ of 0.3 ± 0.1 nM, while insulin glargine and human insulin had an EC$_{50}$ of 2.1 ± 0.5 nM and human insulin 2.3 ± 0.6 nM, respectively, with insulin detemir reported as having the lowest potency of 16.9 ± 2.2 nM (Baus *et al.*, 2010).

There is very little data published regarding the metabolic potential of insulin degludec. According to Nishimura *et al.* (2010) the metabolic responses and maximal effects of insulin degludec were comparable with that of human insulin with respect to lipogenesis in rat adipocytes, glycogen accumulation in rat hepatocytes and glycogen synthesis in rat muscle cells in the absence of albumin.

Insulin-like growth factor receptor-binding characteristics of long-acting insulin analogues

Insulin analogues may have amino acid substitutions and modifications in a domain involved in the interaction with the IGF-1R, and, therefore, may have the potential to display IGF-1-like activities (Werner *et al.*, 2011a). Several independent *in vitro* studies found higher binding affinity of insulin glargine for the IGF-1R compared with human insulin, i.e. ~6-fold higher (Kurtzhals *et al.*, 2000), 10-fold higher (Chisalita & Arnqvist, 2004; Chisalita *et al.*, 2006), 4.6-fold higher (Sommerfeld *et al.*, 2010) and at least 100-fold higher binding affinity of insulin glargine for the IGF-1R compared with IGF-1. In particular, the latter study showed that insulin glargine has a 5.1-fold lower EC_{50} value for autophosphorylation of the receptor. However, the EC_{50} value of IGF-1 was 154-fold lower than for human insulin (Sommerfeld *et al.*, 2010).

The higher affinity of glargine for IGF-1R *in vitro* can be attributed to its additional basic arginine residues at positions 31 and 32 of the B-chain, as demonstrated by the fact that di-$Arg^{B31,B32}$ insulin has an affinity of 2049% for the IGF-1R relative to human insulin. These two arginine residues are also present in human proinsulin and are cleaved during processing to the native insulin molecule. Similarly, these two arginines are rapidly cleaved from insulin glargine once it has been administered *in vivo*. The principle metabolite of insulin glargine, M1, which lacks the B31 and B32 arginine residues, has a lower affinity for IGF-1R than human insulin (42% affinity relative to human insulin) which correlates well with its significantly lower activation of IGF-1R (Sommerfeld *et al.*, 2010).

Insulin detemir has a low affinity for IGF-1R (16% affinity relative to human insulin) (Kurtzhals *et al.*, 2000). In the absence of albumin, the affinity of insulin degludec for IGF-1R has also been described as low (Nishimura *et al.*, 2010).

Insulin-like growth factor receptor phosphorylation and intracellular signalling stimulated by long-acting insulin analogues

It is not clear if and how insulin analogues affect IGF-1R and subsequent signalling differently from regular human insulin, as there are contradictory results in the literature. In HCT-116 colorectal cancer cells, insulin glargine, but not insulin detemir, (both at 50 ng/mL) was shown to phosphorylate IGF-1R (Weinstein *et al.*, 2009). However, a more recent study has shown that in HCT-116 cells, both insulins glargine and detemir phosphorylated IGF-1R in a dose-dependent manner (Yehezkel *et al.*, 2010). With insulin glargine, the maximal IGF-1R phosphorylation was observed at 5 ng/mL. Maximal IGF-1R phosphorylation with insulin detemir was observed at 100 ng/mL, but initial phosphorylation was observed at 25 ng/mL (Yehezkel *et al.*, 2010). At concentrations of 10^{-8} M and 10^{-6} M, insulin glargine was found to phosphorylate the IGF-1R-subunit when tested in human microvascular endothelial cells (Chisalita & Arnqvist, 2004). Thus, supraphysiological concentrations of insulin analogues apparently lead to IGF-1R phosphorylation *in vitro*. Moreover, in cultured cells supraphysiological concentrations of both insulins glargine and detemir stimulated the phosphorylation of ERK (mitogenic signalling pathway) to a similar extent as IGF-1, but human insulin had a significantly greater effect compared with the other ligands (Weinstein *et al.*, 2009; Yehezkel *et al.*, 2010). Whether or not these reports of mitogenic potential at the IGF-1R with long-acting insulin analogues at supraphysiological concentrations in cancer-derived cell lines are of any clinical relevance remains to be seen. Interestingly, in an analysis of serum samples from patients with Type 2 diabetes treated with metformin plus either insulin glargine or NPH insulin, neither treatment increased IGF-1 signalling; indeed, a decrease in serum IGF-1 phosphorylation was observed (Varewijck *et al.*, 2012). However, the potential influence of metformin on these findings cannot be excluded.

Cell proliferation effects with long-acting insulin analogues

One recombinant insulin analogue, Asp^{B10} insulin, which had already been in clinical development, was associated with carcinomas in rat mammary glands (Ebeling *et al.*, 1996). Therefore, it is important to study the effects of all insulin analogues on the insulin/IGF system that may promote cell proliferation. As Asp^{B10} insulin binds and activates IGF-1R *in vitro* this effect was linked to its carcinogenic activity *in vivo*. However, Asp^{B10} insulin also activates IR more potently than human insulin. The mitogenic activity of Asp^{B10} insulin reflects its capacity to activate IR with an increased residence time on the receptor (Berti *et al.*, 1998) as well as potentially activating IGF-1R (Milazzo *et al.*, 1997). Human insulin and the Asp^{B10} insulin analogue cause different patterns of protein phosphorylation when used at physiologically relevant concentrations in MCF-7 breast adenocarcinoma cells, highlighting the importance of understanding the cell proliferation effects of insulin analogues (Oleksiewicz *et al.*, 2011).

Mitogenic activity is often assessed via stimulation of DNA synthesis in proliferation assays (Sandow, 2009). It is important to realize that insulin, as well as IGF-1, belongs to the class of typical growth factors that activate receptor tyrosine kinase signalling pathways in relevant target cells. As a consequence of this, both insulin and IGF-1 trigger cell growth and differentiation, as well as cell-specific metabolism, with insulin having a greater impact on metabolic signalling compared with IGF-1, and IGF-1 being considerably more effective in terms of mitogenic signalling than insulin. However, data regarding the propensity of insulin analogues to promote cell proliferation are heterogeneous and, therefore, difficult to interpret. Some *in vitro* studies showed mitogenic effects in cancerous cell lines (HCT-166, PC-3 [prostate cancer] and MCF-7) with insulins glargine and detemir, but only at very high concentrations (100 nM), whereas IGF-1 has been shown to stimulate cell proliferation by an average of 21% at all doses tested, without any evident dose–response curve (Weinstein *et al.*, 2009). Other studies showed that unmetabolised insulin glargine at 1.5 and 15 nM concentrations had a significantly higher proliferation effect on MCF-7 cells compared with regular insulin, but not on the benign mammary cell line MCF10A (Mayer *et al.*, 2008). Shukla *et al.* (2009) observed enhanced growth of the malignant cell line MCF-7 after stimulation with insulin glargine (0.3 nM) compared with other insulin analogues (both long- and short-acting at concentrations of 1.5–15 nM), but all insulin analogues stimulated the proliferation of MCF-10 cells to a similar level. Shukla *et al.* (2009) also noted that the MCF-7 cells had a higher IGF-1R/IR ratio than the MCF-10 cells. However, another study showed that although insulin glargine stimulated proliferation of both MCF-7 and MCF-10 cells, the level of stimulation was not significantly different from stimulation by human insulin (Staiger *et al.*, 2007). It should be noted that comparisons between these studies should be performed with caution, as different experimental setups and evaluation procedures were used. Insulin-related mitogenic activity is thought to be linked in part to IGF-1R:IR ratio in the cell line studied, with MCF-7 having an IGF-1R:IR ratio of 4:1, whilst the human breast epithelial cell line MCF-10 has a low IGF-1R:IR ratio of 0.8:1 (Müssig *et al.*, 2011).

Neither human insulin nor insulin glargine have been reported to have any effect on the viability and proliferation of human coronary artery endothelial or smooth muscle cells, either at low (0.1 nM) or supraphysiological (100 nM) concentrations (Staiger *et al.*, 2005). Cell proliferation, as assessed by DNA synthesis stimulation, was demonstrated to be similar for primary cultured vascular smooth muscle cells stimulated with equimolar concentrations (1.7, 17 or 170 nM) of human insulin or insulin glargine, and slightly, but not significantly, lower for insulin detemir (Wada *et al.*, 2008). Insulin glargine has also been shown to have a similar mitogenic potency as human insulin in cultured muscle cells and Rat-1 fibroblasts (Ciaraldi *et al.*, 2001; Bähr *et al.*, 1997).

Eckardt *et al.* (2007) studied the effects of insulin and insulin analogues on primary cultured fibroblasts at supraphysiological concentrations (100 nM), showing that regular insulin stimulated DNA synthesis by exclusively activating IR, whereas it appeared that insulin analogues (AspB10; Lantus®, Sanofi; Humalog®, Eli Lilly and Company; and NovoLog/NovoRapid®, Novo Nordisk) mainly signalled through the IGF-1R pathway.

Gly(A21)-insulin, the main metabolite of insulin glargine, was reported to have lower mitogenic potential than human insulin in a human osteosarcoma cell line (Saos/B10) (Kurtzhals *et al.*, 2000). These cells showed a maximum insulin growth response which was >10-fold increased over basal levels. Stimulation of DNA synthesis in these Saos/B10 cells by insulin glargine was approximately 8-fold higher than by human insulin, whereas M1 showed a 3-fold decrease versus human insulin. Assuming that only free insulin detemir is biologically active, the mitogenic potency of insulin detemir relative to human insulin corrected for albumin binding was approximately 11% (10-fold decrease) (Kurtzhals *et al.*, 2000).

Recent findings in a study by Sommerfeld *et al.* (2010) strongly support the idea that insulin glargine metabolites contribute to blood glucose control with the same potency as the insulin glargine parent compound, and with a growth-promoting activity comparable with that of human insulin. Insulin glargine had a more potent stimulation of thymidine incorporation (an indirect measure of DNA synthesis and cell proliferation) in Saos-2 cells, whereas the mitogenic activity of its metabolites M1 and M2 were similar to that of human insulin.

Both insulins glargine and detemir exhibited greater anti-apoptotic effects than human insulin in HCT-116 human colorectal cancer cells, but only at high dose of 100 nM (Weinstein *et al.*, 2009). At a lower dose of 20 nM, human insulin, insulin glargine, insulin detemir and IGF-1 all exhibited a similar anti-apoptotic effect. The mitogenic activity of insulin degludec has been evaluated in myocytes expressing human IR (L6-hIR), human mammary epithelial cells (HMEC), COLO-205 (colorectal adenocarcinoma) and MCF-7 cells, in the absence of albumin, and ranged from 4 to 14% relative to human insulin (Nishimura *et al.*, 2010).

With such a range of evidences available from different cell types, it is difficult to draw definitive conclusions from these *in vitro* findings. Evidence from studies with insulins detemir and degludec show similar or lower proliferative behaviour compared with human insulin at low doses or in albumin-free conditions, as appropriate. It also appears that the insulin glargine metabolites have a lower mitogenic activity in certain cell lines than both the parent compound and human insulin, and this may be the key point for consideration when attempting to translate these findings into an in *vivo* or clinical setting.

In vivo studies on insulin, insulin analogues and carcinogenicity

As discussed above, the Asp^{B10} insulin analogue was found to increase the incidence of breast cancer in rats. The *in vivo* effects of Asp^{B10} insulin, insulin glargine and human insulin (1 IU/kg) on the phosphorylation status of IR, IGF-1R, AKT and ERK1/2 were compared over time in tissue samples taken from rats (Tennagels *et al.*, 2011b; Tennagels *et al.*, 2012). The time courses of the pharmacodynamic effects of human insulin and insulin glargine were found to be distinctively different from that of Asp^{B10} insulin: insulin glargine resulted in phosphorylation levels of IR and AKT that were comparable with that of human insulin. In contrast, injection of Asp^{B10} insulin in rats resulted in at least 2- to 3-fold higher phosphorylation levels and a significantly longer duration of IR and AKT phosphorylation in most of the analysed tissues.

As Asp^{B10} insulin displays higher affinity than human insulin for the IGF1-R *in vitro*, studies were carried out in rats to determine whether insulin analogues with increased IGF-1R affinity *in vitro* also have increased growth promoting activity *in vivo* (Tennagels *et al.*, 2011a; Tennagels *et al.*, 2012). After subcutaneous injection of 1–200 U/kg, no increased IGF-1R autophosphorylation in responsive tissues could be observed, either in response to human insulin or insulin glargine, or even to Asp^{B10}. However, Asp^{B10} insulin, but not human insulin or insulin glargine, induced an increased and prolonged phosphorylation of IR downstream signalling molecules in various tissues. This led the authors to the conclusion that the *in vivo* IR signalling pattern of Asp^{B10} insulin is distinctly different from that of both human insulin and insulin glargine, and that the carcinogenic activity of Asp^{B10} insulin in the rat might be based on its altered IR activation profile and, therefore, be independent from its well-documented increased IGF-1R affinity (Werner *et al.*, 2011b).

Observations from animal models lead to the contention that both diet-induced and genetic hyperinsulinaemia/insulin resistance increases the incidence of aberrant crypt foci (Koohestani *et al.*, 1998; Tran *et al.*, 2003) and chemical-induced colon cancer (Weber *et al.*, 2000; Lee *et al.*, 2001). Furthermore, in rat models of insulin resistance (combined hyperinsulinaemia, hyperglycaemia and hypertriglyceridaemia) the incidence of colorectal epithelial proliferation was also increased *in vivo* (Tran *et al.*, 2006), suggesting that hyperinsulinaemia may augment pro-carcinogenic changes. There is, however, a paucity of conclusive *in vivo* data investigating insulin analogues and carcinogenicity. Mitogenic effects *in vitro* were generally only observed at supraphysiological concentrations in cancer-derived cell lines. Doses of insulin or insulin analogues required to achieve comparably high concentrations *in vivo* would never be applied in clinical practice of as they would result in extreme hypoglycaemia.

Various animal models have been evaluated to determine whether insulin analogues can induce tumours; for example, Stammberger *et al.* (2002) showed in two species over a 2-year period that there was no association between the incidence of specific tumours and insulin glargine (2, 5 or 12 IU/kg SC in rats and mice) or NPH insulin (12.5 IU/kg in mice and 5 IU/kg in rats). There was no difference in the incidence of mammary tumours in both rats and mice when comparing the insulin glargine treatment with either the sodium chloride vehicle-control or the NPH insulin. Furthermore, no consistent dose-dependent increase in the incidence of tumours was observed in either mice or rats, including hepatocellular adenoma or carcinoma, malignant fibrous histiocytoma, mammary gland adenocarcinoma or malignant mammary gland adenoacanthoma (Stammberger *et al.*, 2002). A study by Nagel *et al.* (2010a) directly compared the effects of insulin glargine with NPH insulin on epithelial cell proliferation and aberrant crypt foci formation in colons of *db/db* mice, a commonly used model of type 2 diabetes. In this study, the chronic use (18 weeks) of both insulin glargine and NPH insulin resulted in higher colonic epithelial proliferation and aberrant crypt foci formation compared with a saline control. However, the use of insulin glargine was not associated with increased risk of colonic epithelial proliferation and aberrant crypt foci formation versus NPH insulin. In addition, there was no evidence for lymphadenopathy or spontaneous formation of solid tumours in any treatment group (Nagel *et al.*, 2010a). These results show that suprapharmacological doses of insulin (20–150 IU/kg/day) in the presence of insulin resistance were associated with increased proliferation and aberrant crypt foci formation, irrespective of the type of insulin used, supporting previous evidence in rats showing that hyperinsulinaemia itself enhances colorectal epithelial proliferation *in vivo* (Tran *et al.*, 2006).

Insulin detemir has also been shown to induce proliferative effects in the mammary glands of young female animals. These proliferative effects were described as modest, however, the longest chronic toxicity study with insulin detemir in rats did not exceed 26-weeks (Levemir: EPAR European Medical Association, 2004).

Taken together, these *in vivo* studies illustrate that insulin and all insulin analogues in general, with the exception of Asp^{B10}, share a similar propensity for inducing cell proliferation at supraphysiological doses, which will not be used in clinical practice, but there is currently little evidence to suggest that this translates into tumour development.

Epidemiological data on insulin analogues and carcinogenicity

The findings in epidemiological studies regarding the carcinogenic potential of basal insulin analogues are not consistent, and the interpretation of these studies is limited by confounding factors. Currie *et al.* (2000) and Colhoun (2009) concluded that there was no increased risk associated with insulin analogues compared with human insulin.

A German cohort study by Hemkens *et al.* (2009) reported an association between cancer incidence and insulin dose for all insulin types (human insulin, long-acting insulin analogues and short-acting insulin analogues). Interestingly, the crude incidence rates for cancer and overall mortality were actually lower in the insulin glargine group than in patients treated with human insulin (Hemkens *et al.*, 2009). However, the authors then used an unconventional analysis which found that, after adjusting for dose, the overall incidence of cancer was higher in patients taking insulin glargine compared with human insulin (Hemkens *et al.*, 2009). Nagel *et al.* (2010b) and Pocock and Smeeth (2009) have subsequently both identified a number of limitations in this study, such as flawed statistical methods (including the unconventional analysis), making the data uninterpretable. Furthermore, despite the large variation in mean daily doses observed for the different types of insulin studied, Hemkens *et al.* (2009) failed to take into consideration any pathophysiological reasons for these differences (Nagel *et al.*, 2010b; Pocock & Smeeth, 2009). Patients with either type 1 or type 2 diabetes were grouped together in the study, despite the fact that the two diseases show different patterns of malignancy (Nagel *et al.*, 2010b). Nagel *et al.* (2010b) suggest that data from the German cohort supports previous findings that high doses of insulin, reflecting increased insulin resistance, are associated with an increased risk of cancer and that the data does not warrant safety concerns regarding the use of insulin glargine in diabetic patients.

A nested case-control study showed a possible association between higher insulin glargine doses (≥0.3 IU/kg/day) and cancer incidence in patients with type 2 diabetes (Mannucci *et al.*, 2010). However, in nested case-control studies, the participants from whom the controls were selected may not be fully representative of the original cohort (the control group did not contain those patients who have died from other causes or who have been lost to follow-up); therefore, results from such studies need to be interpreted with caution.

A study by Jonasson *et al.* (2009) also found that women using insulin glargine alone (i.e. with no other types of insulin) had an increased incidence of breast cancer compared with women using types of insulin other than insulin glargine: the risk ratio (RR) over 2 years (2006–2007) was 1.99 (95% CI 1.31–3.03) after adjustment for age and (when appropriate) sex. However, a follow-up to this study by the same group observed no increase in breast cancer during the third year (2008) in patients receiving insulin glargine, leading the authors to suggest that the increased incidence observed in the original publication may have occurred by chance (Ljung *et al.*, 2011).

A number of other observational analyses support the lack of association between insulin glargine use and cancer risk (van Staa *et al.*, 2012; Blin *et al.*, 2012; Lind *et al.*, 2012; Suissa *et al.*, 2011; Boyle *et al.*, 2012; Autier *et al.*, 2012). A recent meta-analysis of data from epidemiological studies involving a total of 907,008 patients and 2,597,602 person-years of observation which included findings from participating centres in the recent Northern European Study reported that the overall risk of cancer (all forms combined) in addition to any organ-specific type of cancer is not increased among glargine users compared with other insulins (Boyle *et al.*, 2012). Based on independent estimates from 13 studies, summary relative risks (SRR [95% CI]) were 0.90 (0.82, 0.99) for all cancers, and 1.11 (1.00, 1.22) for breast cancer. For new users of insulin glargine (based on six studies) SRR of breast cancer was 1.30 (0.93, 1.81). For colorectal cancer and prostate cancer (based on eight studies), SRRs were 0.84 (0.74, 0.95) and 1.13 (1.00, 1.28), respectively (Boyle *et al.*, 2012). Further data from large database studies expected to be released in 2013 (Northern European Study, Kaiser-Permanent Collaboration, the International Study of Insulin and Cancer and a US database analysis), will further elucidate the potential effects of insulin glargine on cancer risk and on the broader insulin-related cancer risk.

Clinical evidence

As previously discussed, studies in patients with diabetes has established that, when administered, insulin glargine is rapidly converted to the active M1 metabolite (Bolli *et al.*, 2011; Lucidi *et al.*, 2011) which has been demonstrated to have low mitogenic activity (Kurtzhals *et al.*, 2000; Sommerfeld *et al.*, 2010). Serum samples from another clinical study in patients with Type 2 diabetes receiving metformin have demonstrated that addition of therapeutic levels of insulin glargine do not increase IGF-1 activity (Varewijck *et al.*, 2012), further supporting the absence of an increased carcinogenic risk.

Randomization overcomes many of the sources of bias that may be associated with observational trials. A randomized, open-label, long-term safety study, designed to assess ocular complications of diabetes, showed that there was no evidence of a greater risk of the development or progression of diabetic retinopathy with insulin glargine versus NPH insulin treatment in patients with type 2 diabetes (Rosenstock *et al.*, 2009a). Although the study was not designed to investigate the effects of treatment on the frequency of tumour development, its long duration allowed the comparative assessment of the occurrence of malignancies with the two treatments (Rosenstock *et al.*, 2009b). In this study, during a 4.2-year follow-up of 1017 patients, there were 20 and 31 patients with incident cancer in the insulin glargine and NPH insulin groups, respectively, indicating that the overall risk of malignancy appears to be similar for both insulins in patients with type 2 diabetes (Rosenstock *et al.*, 2009b). Clinical evidence from the pooled analysis of 31 randomized controlled trials, including over 10,800 people, showed that there was no increased risk of cancer with insulin glargine versus comparator treatments (other insulin types and anti-diabetic drugs) (Home & Lagarenne, 2009). Another meta-analysis of randomized controlled studies of at least 12 weeks' duration showed that there was no increased risk of malignancies in patients with diabetes treated with insulin detemir compared with NPH or insulin glargine (Dejgaard *et al.*, 2009). Overall, the incidence rate of cancer was small for all groups, probably reflecting the inclusion/exclusion criteria typical for study populations designed for achieving regulatory approval and the short duration of the follow-up period (Dejgaard *et al.*, 2009).

This is supported by randomized, controlled trial data from the ACCORD (Action to Control Cardiovascular Risk in Diabetes) and ORIGIN (Outcome Reduction with Initial Glargine INtervention) studies. An analysis of 5-year data from 10,251 patients with high cardiovascular risk in the ACCORD study was powered for cardiovascular outcomes but also investigated cancer-related outcomes (Hamaty *et al.*, 2011). The authors concluded that exposure to any insulin, or to basal insulin or insulin glargine specifically, was not associated with increased risk of cancer-related outcomes (hospitalization or death); hazard ratio (HR) related to insulin glargine was 1.00 (95% CI: 0.53, 1.86), $p = 0.99$ (Hamaty *et al.*, 2011).

The ORIGIN study, which investigated insulin glargine versus placebo treatment in patients with high risk of cardiovascular events and early Type 2 diabetes or prediabetes (impaired fasting glucose or impaired glucose tolerance) also included cancer incidence as a secondary outcome (The ORIGIN trial investigators, 2012). Findings from this study of 12,537 patients followed for a median of 6.2 years reported no increased incidence of all cancers combined, any organ-specific type of cancer (including breast, lung, colon, prostate, melanoma) or cancer mortality, in the insulin glargine group versus the standard care group. Hazard ratio (95% CI) for the incidence of all cancers was 1.00 (0.88, 1.13), $p = 0.97$ and for death from cancer was 0.94 (0.77, 1.15), $p = 0.51$ (The ORIGIN trial investigators, 2012). To our knowledge, there are no published data regarding the use of insulin degludec and the risk of malignancies. Further analyses from the ACCORD and ORIGIN studies may help elucidate any potential effects of insulin glargine on the incidence of cancer in a randomized, controlled, clinical trial setting.

The increased affinity some insulin analogues may have for IGF-1R raises concerns that if insulin analogues are used during pregnancy, they might lead to increased foetal growth and other mitogenic effects (Pollex *et al.*, 2011). A meta-analysis comparing insulin glargine with NPH insulin in pregnant women showed that there was no increased risk of congenital abnormalities with insulin glargine (risk ratio [RR] 0.97; 95% confidence intervals [CI] 0.47–1.99), macrosomia (>4 kg; RR 1.28; 95% CI 0.77–2.12) or babies born large for gestational age (>90th centile; RR 1.02 95% CI 0.80–1.31) (Pollex *et al.*, 2011). However, seven of the eight studies included in this meta-analysis were retrospective in nature and all were observational, thereby limiting the general applicability of the results. No randomized controlled trials of long-acting insulin analogues in pregnant women have yet been conducted. Evidence using an *in vitro* human placental perfusion model designed to demonstrate the rate of transfer across the human placenta showed that at therapeutic concentrations no insulin glargine was detectable in the foetal circuit (Pollex *et al.*, 2010).

Human IGF-1 has also been tested in clinical studies (including Phase III studies) of type 2 diabetes for potential practical and physiological advantages versus insulin (Zenobi *et al.*, 1992). When given to patients with type 2 diabetes for 5 days, IGF-1 has been shown to improve fasting and postprandial glycaemia and to decrease triglyceride values as well as to increase insulin sensitivity (Zenobi *et al.*, 1992). The risk of hypoglycaemia in type 2 diabetes has also been reported to be significantly lower with IGF-1 than with conventional insulin therapy. Based on these initial reports, it has been suggested that IGF-1 therapy may have a role in treating severely insulin-resistant patients unresponsive to currently available forms of insulin therapy. Relying on these early promising studies, patient safety, with regard to potential IGF-1-mediated proliferation of endothelial and smooth muscle cells, as well as growth-promoting effects on various carcinomas, has been investigated and found to be not significantly affected during IGF-1 treatment.

Conclusions

Although some insulin analogues have demonstrated mitogenic potency in various *in vitro* studies in cancer cell lines, none of the currently commercially available insulin analogues has yet been proven to be mitogenic *in vivo,* neither in animals nor in humans. In the case of insulin glargine, this can be attributed to its rapid metabolism into metabolites that have no greater mitogenic activity than human insulin. There is no evidence that either human insulin or insulin analogues have carcinogenic effects at therapeutic doses. Furthermore, in clinical practice, the supraphysiological concentrations required to promote tumour growth *in vitro* will not be achieved in patients owing to the risk of hypoglycaemia. Taking this into account, there is also no evidence from *in vitro* studies to support the hypothesis that currently marketed insulin analogues promote tumour growth in humans when used in therapeutic doses. The ORIGINALE (ORIGIN *A*nd *L*egacy *E*ffects) study, in addition to long-term findings from other observational studies, will clearly establish a deeper knowledge of the relationship between diabetes, the incidence of malignancies and the safety of long-acting insulin analogues.

Declaration of interest

Norbert Tennagels and Ulrich Werner are employees of Sanofi-Aventis Deutschland GmbH. Editorial support was provided by Huw Jones, PhD, and Róisín O'Connor, PhD, of Medicus International and funded by Sanofi.

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