Insulin Therapy in Diabetes and Cancer Risk: Current Understanding and Implications for Future Study

Proceedings from a meeting of a European Insulin Safety Consensus Panel, convened and sponsored by Novo Nordisk, held Tuesday October 5, 2010 at The Radisson Edwardian Heathrow Hotel, Hayes, Middlesex, UK

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Received: May 27, 2011 / Published online: August 19, 2011
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ABSTRACT

Introduction: Interest in the possibility of certain insulin treatments having the potential to modify cancer development and prognosis was reawakened in 2009, following publication of several epidemiological studies addressing this issue. This interest extends to how diabetes itself and cancer might be linked, and makes desirable an exchange of expert views and knowledge to enhance understanding in this

subject among those treating diabetes and cancer, or those developing diabetes therapies.

Methods: A European meeting was convened with participants invited based on known relevant interests in endocrinology, oncology, epidemiology, and insulin analog design and investigation. Experts in these fields were invited to present on relevant topics, with open discussions held after each presentation.

Results: Concern over the potential mitogenic properties of certain insulin analogs has

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arisen from some (but not all) epidemiological studies, although confounding factors render interpretation controversial. Future epidemiological studies are likely to strengthen confidence in drawing conclusions. Meanwhile, pharmacological studies, and a consideration of cancer pathophysiology, implicate increased insulin-like growth factor-1 receptor affinity, and/or deranged insulin receptor interaction/signaling properties as possible *a priori* causes for concern with some insulin analogs. Again, interpretation of the body of pharmacological evidence is confounded by the array of test systems and methodologies used, and by studies frequently succumbing to methodological pitfalls. Reassuringly, most available insulin analogs do not differ in their receptor interaction response profile to human insulin, and for those that do there are reasons to question any potential clinical relevance. Nevertheless, it is desirable that new experimental models are devised that can better determine the likely clinical consequences of any variance in receptor response profile versus human insulin. **Conclusion:** More data are required to increase our understanding of this issue. To facilitate and disseminate such understanding, close cooperation and communication between diabetologists, epidemiologists, oncologists, and insulin engineers will be essential.

Keywords: cancer, detemir, diabetes, glargine, insulin, insulin-like growth factor, insulin receptor, mitogenicity

INTRODUCTION

Stephen Gough, Meeting Chairman

The disclosure of data in June 2009 of four epidemiological studies,¹⁻⁴ three of which reported a potential link between the use

of insulin glargine and an increased risk of cancer,^{1,3,4} has provoked ongoing debate about the long-term clinical safety of insulin analogs. This debate arises because of anomalies and confounding factors in these four retrospective studies that cast doubt on the conclusions reached. Type 2 diabetes mellitus (T2DM) is itself associated with an increased incidence and risk of mortality from a range of cancers,^{5,6} and associated comorbidities and lifestyle factors, such as obesity and physical inactivity, are also associated with increased risk.⁷ Furthermore, different diabetes treatments (which are often used concomitantly) can modify the risk of developing cancer (with metformin, for example, providing a protective effect^{8,9}) and insulins and sulfonylureas potentially promoting tumor growth.^{10,11} This intricate web of interactions makes interpretation of epidemiologic studies difficult and, perhaps unsurprisingly given the controversial and far-reaching nature of the topic, the 2009 Diabetologia papers drew appropriate criticism over methodology.¹²

In response, the European Medicines Agency has now listed insulin analog and cancer safety as one of its priorities for drug safety research in 2011.¹³ The insulin manufacturer Novo Nordisk (Copenhagen, Denmark) is one of several companies that has an interest in this field, and in ensuring that the molecular safety profile of insulin analog products is both satisfactory and established through appropriate scientific study. Novo Nordisk, therefore, convened and sponsored a meeting of individuals with expertise and/or research interests in this area to enable free exchange of views and information. This group was designated 'The European Insulin Safety Consensus Panel,' as it was hoped that, in addition to providing a timely update on results from recent and ongoing studies, the attendees would assess the possibility of

reaching consensus on how to interpret the available data and the next steps required in future research. The panel members listed as authors gave presentations at the meeting on topics suggested by the sponsor, although the content of these presentations was not influenced by the sponsor. Here, the views and data presented, and salient discussion points arising, are summarized. The subheadings below reflect the titles of the presentations given, but in the interest of keeping topics coherent for the reader, some slight modifications have been made in this report to the original sequence of presentations.

THE INSULIN AND CANCER STORY - HOW IT ALL STARTED

Christopher Poole

How diabetes and its treatment affect cancer risk and outcome has been studied steadily over the last decade (driven primarily by oncologists). The possibility that insulin analogs could affect cancer development differently to human insulin was first realized with the development of the X10 analog, as described below. However, four epidemiological papers published in *Diabetologia* in 2009¹⁻⁴ have greatly increased awareness among the diabetes community that widely used treatments might influence patients' risk of cancer. As a result, publications on the subject in diabetes journals have multiplied over the last year, and the issue has drawn the attention of many diabetologists for the first time. The story of these publications in *Diabetologia* was somewhat controversial, and there remains disagreement about how best to interpret the data. Indeed, the original German study submission by Hemkens and colleagues¹ presented a challenge to the journal referees (due to methodological concerns) and

gave the *Diabetologia* editors a moral dilemma, which they resolved by commissioning three validation studies using databases in the UK, Sweden, and Scotland.²⁻⁴

In brief, the Hemkens study described a crude incidence of cancer that was higher with human insulin than with insulin analogs.¹ After adjusting for dose, however, insulin glargine was associated with an increased incidence of all cancers. Conversely, short-acting insulin analogs did not appear to be associated with excess cancer or mortality. The UK study by Currie and colleagues² found that insulin and sulfonylurea therapy was associated with an increase in risk of pancreatic and colorectal cancer, but not of breast cancer. Combination therapy involving metformin protected against the increased cancer risk with these therapies. There was no difference in cancer risk between specific insulin regimens in this study. The Swedish study³ found that women using insulin glargine alone (ie, without other insulin products, although not necessarily as their sole diabetes therapy) had an increased incidence of breast cancer, but other malignancies did not appear to be affected by glargine. The Scottish study⁴ found no difference in total or site-specific cancer in those exposed or not exposed to insulin glargine. However, patients exposed to glargine alone had a higher rate of all cancers, including breast cancer.

Such epidemiological studies are always open to competing ways of interpretation, but various methodological weaknesses in the original Hemkens study should be considered. First, there was no differentiation made between type 1 (T1) and T2DM, and no adjustment made for body mass index or endogenous insulin status; hence the comparator cohorts are likely to have had different demographics. This problem is also highly likely to pertain to the analysis of 'glargine alone versus any other insulin regimens' used in the Scottish⁴ and Swedish³

studies. Second, in the primary analysis of the German study,¹ glargine carried a lower relative risk than other insulins, which reversed only after adjustment for dose. It is usual practice to adjust for potential confounding factors to see if an association found with raw data disappears after adjustment, but not to identify new associations. With the latter approach, any result might be claimed if repeated adjustments are made until an expected finding is seen. Adjusting, it should be remembered, is not the same as controlling. Third, when data were stratified by three dose groups, a U-shaped curve was observed, which seems implausible, and the follow-up period (1.3 years) seems very short for a cancer risk difference to manifest. Finally, the *a posteriori* treatment group allocation based on follow-up information and the use of mean insulin doses observed during the follow-up period, as baseline covariates in a survival analysis, are fundamental errors in an epidemiological analysis.

Discussion Points Arising

Future epidemiologic studies would ideally take account of insulin resistance as a confounding factor, as high insulin doses are typically associated with major insulin resistance. In practice, however, this would be difficult to do.

The data from the Hemkens study might have been more convincing had the approach been to compare glargine-only to Neutral Protamine Hagedorn (NPH)-only insulin therapy. However, there was some logic to the approach taken, as it reflected the historical use of insulin in T2DM in Germany: the tradition has been to use supplementary mealtime insulin therapy, with basal insulin plus oral antidiabetic drug therapy only recently gaining favor as an alternative regimen.

There are at least three possible explanations as to why an elevated cancer risk might manifest

so soon after insulin initiation. First, it is possible that the sudden rise in insulinemia activates latent insulin-sensitive neoplastic loci. Second, an alternative (but not mutually exclusive) possibility is reverse causality, ie, a pre-existing tumor that affects glycemia might lead to insulin initiation. In this respect, diabetes and liver cancer are certainly related via hepatitis C infection. A third possible reason why an excess of cancer appears to manifest 'early' in the natural history of diabetes is that any potential excess of later-life cancer is subjugated by cardiovascular mortality.

Ideally, future epidemiologic studies should be based on a consensus of good methodology to cover:

- adequate accounting for competing cancer risk factors
- comparisons based on well-matched groups with equivalent exposures
- the use of agreed covariates in statistical analyses
- cancer heterogeneity.

RECENT EPIDEMIOLOGIC FINDINGS FROM ITALY AND FINLAND ON THE RISK OF CANCER ASSOCIATED WITH DIABETES AND HYPERGLYCEMIA

Edoardo Mannucci · Jaakko Tuomilehto

In an effort to address many of the confounders of the 2009 Diabetologia publications, an Italian study was conducted that employed a nested case-control design.¹⁴ This considered long-term follow-up data (6.3 years) from a consecutive series of 1340 out-patients with T2DM initiating insulin therapy and with no previous malignancies. Incident cancer cases ($n=112$) were identified through hospital discharge records and death registries. Comparisons were

made between cases and matched controls to identify predictors of incident cancer. The use of glargine was not found to be higher in cases than controls, but cases using glargine were taking higher doses than controls using glargine; a relationship that did not apply to other insulins. After adjusting for comorbidities, other types of insulin, and metformin exposure, there was an increased risk for incident cancer in patients taking glargine at doses ≥ 0.3 IU/kg/day (odds ratio: 5.43 [95% confidence interval: 2.18-13.53], $P < 0.001$), both when taking the overall observation period into consideration and when excluding all incident cancers from the first year of glargine treatment. Metformin was under-represented in cases versus controls, supporting other work suggesting a protective effect.^{2,15,16}

Another study, yet to be published, that offers considerable promise for informative data is being conducted in Finland and involves computer linkage between the entire national diabetes database (all patients diagnosed between 1988-2007), and cancer and other registry databases. Age at diagnosis and drug therapy will allow a reasonably reliable classification into T1DM and T2DM categories. The number of observed cancer cases can be compared with national averages to generate standardized incidence ratios, stratifying patients (including children) into 5-year age interval groups, and calculating person-years of risk for calendar periods between 1988 and 2008. Preliminary analyses of the data reveal significant increases (of more than 10%) in the overall cancer risk of people with diabetes versus the background population. More than 20 different site-specific cancers were also found to be significantly increased in people with diabetes. However, one issue, highlighted by a recent Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe (DECODE) group study,¹⁷ is that cancer risk associated with

diabetes may be underestimated due to the risk extending 'down' into the undiagnosed hyperglycemic population. Incident cancers in these individuals are currently categorized as occurring in the 'normal' population.

Discussion Points Arising

The Finnish study, alone or in combination with similar data from other countries, may be of sufficient size and scope to enable construction of matched groups to study outcomes by exposure to different diabetes therapies, to undertake time-to-event analyses, to investigate the incidences of subtypes of breast cancer, and to investigate the influence of a family history of cancer.

Frequent contact with care providers might inflate the cancer diagnosis rate in people with diabetes. It could be useful to make comparisons with cohorts of people without diabetes, but who also have regular contact with carers.

There is a paradox that prostate cancer seems to be less frequent in men with diabetes than in the background population. Possible explanations include hypogonadism associated with obesity and the difficulty of palpating a prostate lesion in an obese man. This finding from several epidemiologic studies is supported by a reduction in the diagnostic prostate-specific antigen in men with T2DM.^{18,19}

THE IGF SYSTEM: OVERVIEW OF ITS ROLE IN TUMORIGENESIS

Matthias Weber

The mitogenic influence of insulin mediated via the insulin-like growth factor (IGF) system is currently considered as one of the most plausible mechanisms to explain a link between insulin use and cancer risk. This system encompasses a

complex regulatory network that includes two peptide growth factors (IGF-1 and IGF-2) with homologies to insulin, cell-surface receptors for these peptides (including IGF-1R and IGF-2R), and six high-affinity IGF binding proteins (IGFBP), to which most circulating IGF is bound. In mammals, IGF-1 is produced in large amounts in the liver in response to pituitary growth hormone (GH). It is also produced locally as a paracrine/autocrine growth factor. IGF-1 interacts with IGF-1R to stimulate cell growth and mitosis, and IGF-2 has a high affinity for both IGF receptors and acts as an important fetal growth factor. IGF-2R does not appear to produce a signal, but instead acts to degrade IGF-2 and as a tumor suppressor.

The IGF system appears to have evolved to regulate somatic growth of the organism, coupled tightly to nutritional status, and in many animals this regulation extends to reproduction and longevity.²⁰⁻²³ Indeed, both calorie restriction and disruption of IGF signaling extend lifespan in many species, ranging from worms and flies through to mammals.^{20,21} Interestingly, mutations in IGF-1R (resulting in decreased functional activity) are more prevalent in centenarian humans.²⁴ The importance of the IGF system for survival is demonstrated by the fact that it is highly conserved between species throughout evolution, particularly the gene encoding IGF-1.²⁴

However, what role does the IGF system play in cancer? Various epidemiologic studies have shown a correlation between high circulating levels of IGF-1 and an increased risk of breast, colorectal, lung, and prostate cancer.²⁵⁻²⁸ Conversely, there tend to be negative associations between serum IGFBP-3 and cancer risk.²⁶⁻²⁸ IGFBP affect signaling by sequestering IGFs (or, in some cases, presenting IGFs to receptors), which are themselves regulated by IGFBP-proteases. IGFBP may also bind cell surface receptors to directly affect cellular processes.²⁹

Acromegaly provides a potential model to study the influence of the IGF system on prognosis, as IGF-1 levels are raised in response to GH hypersecretion. The available epidemiologic data suggest only a small excess of cancer in people with this condition, compared with the general population.³⁰ This observation does not implicate IGF as being carcinogenic, although it may drive the growth of pre-existing, subclinical tumors. This hypothesis is supported by the observations that (along with overexpression of IGF-2), many cancers over-express intact IGF-1R, and that inactivation of the IGF-1R, for example after transfection of a dominant negative IGF-1R, can retard cancer cell growth experimentally.³¹

Other observations, however, suggest that IGF can malignantly transform some cells: fibroblasts require the presence of intact IGF-1R to undergo malignant transformation via the transforming action of oncogenes.³² Also, the over-expression of IGF-1R can induce malignant transformation in some cell models, eg, NIH 3T3 cells.³³

Cancer cells frequently over-express IGF-1R, IGF-2, and (sometimes) IGFBP-2. It is thought that these abnormalities usually occur late in the natural history of neoplasia and accelerate the growth of transformed cells. Therefore, various agents that inhibit IGF-1R signaling are in development as putative anticancer therapies.³⁴

While many tumor types over-express IGF-1R, others also over-express insulin receptor (IR), and over-expression of each receptor type in most cases is predictive of poor prognosis.³⁵ Although these receptors exhibit distinct roles in normal physiology (IR activation resulting primarily in metabolic outcomes and IGF-1R activation leading mainly to mitogenic outcomes) there is some overlap in the downstream processes they are able to effect. There is also some binding affinity between the insulin molecule and IGF-1R (and between IGF-1 and IR), albeit that

each ligand has a much higher affinity for its own receptor. It is therefore unsurprising that insulin can also influence tumor development and progression; indeed, mitogenic effects with insulin were first demonstrated *in vitro* in the 1950s.^{36,37} Although the mitogenic potency of insulin is much less than that of IGF-1, insulin can alter cell responses to cellular growth factors via activation of the farnesyltransferase pathway, enhancing the mitogenic potential of, among other growth factors, IGF-1.³⁸ Furthermore, hyperinsulinemia has been associated with increased tumor proliferation, and the growth of some human breast cancer cells has been shown to be responsive to insulin.^{39,40}

The IR exists as two isoforms, and cancer cells tend to express the IR-A isoform, which is normally characteristic of placental and fetal tissues. IR-A also has a high affinity for IGF-2 and can evoke mitogenic responses. IR-A expression tends to occur late in the natural history of tumors so is unlikely to be causative, but it might be permissive in tumor growth. To complicate matters further, cancer cells can also express IR/IGF-1R hybrid receptors and the relevant ligands (insulin, IGF-1, IGF-2) have some affinity for these hybrids too, which can again evoke mitogenic responses.

Discussion Points Arising

Changes in insulin/IGF-1 function at a cellular level may offer a survival advantage to latent neoplasms by facilitating growth signal self-sufficiency and resistance to apoptosis.⁴¹ It is also possible that the signaling of IR and IGF-1R might be modified by the metabolic conditions associated with diabetes and its therapy, such as periods of hypoglycemia. Similarly, hyperinsulinemia has an important influence on cancer risk and it is possible that IR-mediated metabolic pathways (and tissues)

become insulin resistant in diabetes, whereas mitogenic pathways remain insulin sensitive. Furthermore, the role of IR/IGF-1R hybrids and the fetal isoform of IR in tumorigenesis need to be elucidated in future studies.

INSULIN ANALOGS, IGF-1R, AND STEM-LIKE CANCER CELLS

Cristóbal Belda-Iniesta

Relevant to the consideration of insulin analog development is the fact that genes encoding the IGF-1 and insulin molecules are homologous, having arisen, it is thought, from a common ancestor.⁴² Consequently, human IGF-1 and insulin molecules share 45% amino acid homology. There is also considerable (~60%) homology between IR and IGF-1R. IGF-1 differs from insulin by an additional three amino acid residues at the B terminus (two arginines interspersed with a tyrosine) that introduce a positive charge. This draws the peptide into the negatively charged external binding pocket of the IGF-1R dimer.

Given these close similarities, it is possible that modified insulin molecules could inadvertently have increased IGF-1R affinity. This is of potential clinical concern because of the tumor-promoting (transformation and mitosis) effects mediated via IGF-1R elaborated in the previous presentation. IGF-1R activation is also associated with poorer cancer prognosis,^{43,44} increased rate of metastasis,⁴⁵ and reduced responsiveness to radiotherapy,^{46,47} chemotherapy,^{43,48-50} and targeted therapies against the endothelial growth factor receptor.^{49,50}

However, even if an analog has increased IGF-1R affinity, it does not automatically follow that this will be of clinical consequence. Firstly, there is the consideration that the absolute IGF-1R affinity relative to human insulin may

still be orders of magnitude lower than that of IGF-1 itself. The proliferative potential of such an analog may manifest when added in isolation to the culture medium of cells expressing IGF-1R, but such test systems could be considered 'worst case scenario,' as *in vivo* cancer cells are likely to express a mixture of receptor types and to meet a host of potential ligands including native IGF-1. The question arises of whether an analog with an elevated IGF-1R binding affinity would even reach cancer cells in sufficient concentrations to evoke proliferative responses, and whether such a signal would be 'drowned out' by endogenous IGF-1 anyway. Against this possibility is the fact that most circulating IGF-1 is bound by IGFBP, and hence is biologically inactive. It is known that interstitial fluid pressure can be increased in tumor tissue compared with healthy tissue, thereby presenting a potential barrier restricting the access of circulating peptide hormones; both exogenous and endogenous.⁵¹ A further potential barrier may be presented by increased local collagen deposition. Thus, the endocrine environment may be of little importance compared with paracrine/autocrine responses within the developing tumor.

Another area of uncertainty for consideration is that of insulin analog metabolism. Insulin glargine incorporates two additional arginine residues at the B chain terminus, giving this region a positive charge, as is the case with IGF-1, and hence an increased affinity for IGF-1R. However, these residues can be enzymatically cleaved to produce two metabolites that do not have elevated IGF-1R affinity.⁵²

In summary, IGF-1R can be considered a proto-oncogene and the inhibition of its signaling is now being studied as a therapeutic target in oncology. Despite some continuing uncertainties, there are therefore theoretical reasons for avoiding the development of insulin analogs with increased IGF-1R

affinity based on modifications that are not enzymatically cleaved.

Discussion Points Arising

It was noted that the evidence that insulin glargine is metabolized comes from an *ex vivo* study⁵² in which glargine was cultured with human blood for 30 minutes; much longer than the circulating half-life of the drug. Therefore, speculation remains as to whether a high percentage of glargine is metabolized *in vivo*. Of potential relevance here, Mayer and Chantelau⁵³ added serum taken from patients treated with glargine to the culture medium of human breast cancer cells. In contrast to the serum of patients treated with NPH or insulin detemir, this was associated with a statistically significant 11% increase in proliferative response, but it should be noted that this study was not controlled for other components of human sera.

The issue was raised of the timescale, over which endocrine factors can influence cancer risk manifestation, and it was noted that the increased risks associated with hormone replacement therapy and the contraceptive pill normalize within 5 years of discontinuation. This suggests that endocrine influences on cancer incidence can occur in shorter time-scales than popularly appreciated.

INSULIN MODIFICATION AND CONSEQUENCES FOR MITOGENICITY

David Russell-Jones

The realization that artificial modifications of the insulin molecule could sometimes lead to unintended increases in mitogenic potential (ie, the ability of the analog to stimulate cell division at least *in vitro* to a greater extent than human

insulin) dates back to the early 1990s when the insulin manufacturer Novo Nordisk developed a rapid-acting insulin analog called 'X10'. This monomeric analog differed from human insulin by a single amino acid substitution, with the B10 histidine residue replaced with aspartic acid, to create a peptide that occurs rarely as a natural mutation.^{54,55} In pharmacokinetic/pharmacodynamic studies, subcutaneously injected X10 more closely mimicked the natural prandial insulin response than soluble human insulin,⁵⁶ but clinical development was terminated when toxicology studies revealed a dose-related increase in incident mammary tumors in female Sprague-Dawley rats.⁵⁷ This then prompted a series of studies designed to identify the possible mechanism(s) responsible, and X10 was found to differ from human insulin in two important pharmacological properties (Figure 1).^{58,59} Firstly, X10 was found to bind^{59,60} and activate⁶¹ IGF-1R with greater potency than that of human insulin. Thus, the ratio of binding affinity for IGF-1R relative to IR was increased about four-fold. Secondly, X10 occupied (and stimulated) the IR for a longer period of time than human insulin before internalization and degradation of the ligand-receptor complex, ie, X10 had a reduced IR 'off-rate'.^{58,62,63} Importantly, by constructing a series of experimental insulin analogs that varied

in each of these two properties, it was shown that the ratio of IGF-1R:IR binding affinity^{59,64} and the IR residency time^{58,59} of insulin analogs each correlate with mitogenic potency in cell models expressing the relevant receptors. The evidence suggested that the relationship between relative IGF-1R affinity and mitogenic potency is continuous, whereas increased mitogenic potency only manifests when IR off-rate declines below ~40% that of human insulin.⁵⁸ Thus, the analog development program at Novo Nordisk, where X10 had been constructed and studied, incorporated the criteria that any analog to be brought into clinical development should have an IGF-1R:IR affinity that does not exceed that of human insulin, and should have an IR off-rate not markedly lower than that of human insulin.

Whether IGF-1R affinity is of greater concern than increased IR residency time is unknown, but one *in vitro* study has suggested that the IGF-1R-mediated proliferative effect predominates in human fibroblasts.⁶⁵ Here, silencing of IGF-1R significantly blunted ligand-mediated stimulation of Akt phosphorylation by IGF-1, X10, and insulin glargine (by 72%, 58%, and 40%, respectively), while the insulin-mediated response remained unaffected. In contrast, silencing of IR did not significantly alter Akt phosphorylation in response to IGF-1, X10, or glargine.

Figure 1. (A) Insulin receptor (IR) off-rate and (B) insulin-like growth factor (IGF-1) receptor:IR binding affinity for various insulin-like ligands, with values expressed as percentages of human insulin. *Adapted with permission from Kurtzhals et al. Diabetes 2000.*

Two epidemiological studies have been published based on insulin analog manufacturers' databases. A meta-analysis of 31 randomized controlled clinical trials, comparing insulin glargine with any comparator in T1DM or T2DM,⁶⁶ did not find an increased risk of cancer with glargine versus comparator treatment in any study, with the comparator being NPH insulin in 21 studies. A second meta-analysis of 16 randomized trials (>12 weeks) involving 8693 patients with T1DM or T2DM compared insulin detemir with either NPH or insulin glargine.⁶⁷ Here, insulin detemir was not associated with an increased risk of cancer compared with NPH or glargine (events/100 years: 0.36, 0.92, and 1.27, respectively; not significant).

Discussion Points Arising

The greatest limitation of the glargine and detemir meta-analyses was the short time-scale of the included studies. The Outcome Reduction with Initial Glargine Intervention (ORIGIN) study⁶⁸ will provide data for >10,000 patient-years of exposure to glargine in the absence of metformin, but even this might be underpowered to detect even a 30% difference in cancer risk. Of course, this study was not designed to evaluate this endpoint. A large-scale International Strategic Cancer Alliance study on breast cancer might also provide new data on associations with diabetes and insulin use.

PRINCIPLES FOR INSULIN ANALOG DEVELOPMENT

Bo Falck Hansen

The hypothesis that mitogenesis might at least in part be mediated via IGF-1R binding, and early reports⁵⁹ that insulin glargine had an approximately six-fold increase in affinity for

IGF-1R (and an approximately nine-fold increase in IGF-1R:IR affinity ratio) compared with human insulin led to the original epidemiologic enquiry by Hemkens et al¹ and the associated confirmatory studies.²⁻⁴ These in turn have led to a spate of more recent *in vitro* studies revisiting the receptor binding and mitogenic properties of insulin analogs, and applying different models and methodological techniques. However, to interpret the findings of these studies, it is important to consider some principles of investigation that have been previously highlighted,⁶⁹ but which have been overlooked in several *in vitro* studies.

Firstly, it is important to realize that the relative potencies of different insulin analogs for given metabolic or mitogenic effects can only be determined from full dose-response curves.⁶⁹ An insulin-like molecule is likely to have at least some affinity for both IR and IGF-1R, so is potentially able to exert anything from zero to full response in cells carrying one or both receptor types, depending on its concentration (Figure 2).⁶⁹ Thus, comparisons of different

Figure 2. Representative dose-response curves, with shapes typical of insulin-like ligands. Black dotted double-headed arrow indicates the constant difference between two ligands of high (solid black) and low (dotted black) potency across the responsive part of the dose-response curve. Grey arrows illustrate how the use of fixed doses could be misconstrued to suggest either a large (left) or small (right) response difference between the ligands. Adapted with permission from Pollak & Russell-Jones, *Int J Clin Pract.* 2010.⁶⁹

ligands made at either very high doses (at which they are all exerting a maximum effect), or very low doses (at which they are exerting little or no effect) are unlikely to show differences, and consequently have little value. Instead, full dose-response curves should be constructed. If the curve is shifted to the left relative to human insulin (ie, the response commences and maximizes at lower concentrations), then this ligand has greater potency for the response in question. Conversely, a rightward shift indicates reduced potency. Relative potencies can then be compared in terms of median effective concentration (EC50) values.

Potency alone, however, is still insufficient to indicate the mitogenic potential of an analog *in vivo*, as insulin-like ligands vary greatly in their absolute affinities for receptors. Therefore, even EC50 values from full dose-response curves are potentially misleading and, when considering the potential for IGF-1R-mediated mitogenicity in receptor-binding studies, what is important is the ratio of effect that a ligand has for IGF-1R:IR binding/activation potency or its mitogenic:metabolic potency and how this ratio compares with human insulin (Figure 1). These ratios might determine the likely *in vivo* proportions of binding among the various potential receptors available to the ligand, and hence the likely biologic effects in the organism.

Another pitfall when investigating mitogenicity in *in vitro* experimentation is to use cell types that are poorly responsive or (worse) do not carry appropriate receptors. Such cells will inevitably fail to show differences in mitogenic response between one ligand and another, and could give false reassurance. Suspicion is called for if positive controls, such as the known mitogens X10 and IGF-1, fail to show substantially greater mitogenic responses than human insulin and/or the study ligands (Figure 3).^{70,71}

Figure 3. Importance of cell type, receptor expression and ligand dose in evaluating mitogenic potency of insulin analogs. (A) No difference in proliferation is seen between insulin analogs across a range of doses when they are added to the culture of cells expressing relatively few insulin-like growth factor receptor 1 (IGF-1R), whereas (B) insulin glargine shows an increased proliferative response in cells expressing relatively many IGF-1R. MCF=Michigan Cancer Foundation. *Reproduced by permission from Shukla et al. 2009*⁷⁰ © Society for Endocrinology 2011. (C) Embryonic mouse fibroblasts expressing IGF-1R and exposed to a single concentration of the study ligands (5 nmol/L for all except insulin detemir given at 19 nmol/L). Note that IGF-1 evokes only an approximately 30% greater response than human insulin at this dose in this cell system. *Reproduced with permission from Sciacca et al. Diabetologia. 2010. Springer Science+Business Media.*⁷¹

If the hypothesis is that analogs with an increased IGF-1R:IR affinity relative to human insulin might accelerate tumor growth, then testing this will require use of cells expressing abundant IGF-1R that respond by exhibiting proliferation. Predictably, those studies that have not shown differences between insulin glargine and other insulins in terms of binding affinities and/or mitogenic effects have tended to use incomplete dose-response curves⁷¹⁻⁷³ and/or unresponsive cell lines, in which IGF-1 itself showed relatively little increase in potency/effect size versus the comparators or human insulin.⁷¹⁻⁷⁵ In contrast, studies using complete or near-complete dose-response curves and/or responsive cells expressing IGF-1R have tended to confirm the earlier work of Kurtzhals and colleagues⁵⁹ in suggesting that insulin glargine has a greater IGF-1R:IR affinity ratio than human insulin, with greater mitogenic potency mediated via IGF-1R.^{70,76-78}

One study by Hansen and colleagues⁷⁸ has been undertaken in an attempt to redress the inconsistencies of other recent publications, by constructing full dose-response curves to make a comprehensive series of comparisons of insulin glargine, insulin detemir, and human insulin with regard to receptor interactions and mitogenic responses. This study compared the ligands' IR and IGF-1R binding affinities, regional receptor phosphorylation, and receptor activation kinetics, as well as mitogenic potential in two different cell types chosen because they predominantly express either IR or IGF-1R. The study also examined the ligands' relative affinity and activation of IR-A and IR-B. This is significant because it can be speculated that a greater potency for IR-A versus IR-B activation (relative to human insulin) could also increase the mitogenic risk associated with an insulin analog.^{71,79} In summary, this work confirmed earlier studies by the groups of Kurtzhals⁵⁹ and Sommerfeld,⁷⁶ by showing that insulin

detemir was not associated with an increased IGF-1R:IR binding ratio, or prolonged IR residency, whereas insulin glargine displayed an increased IGF-1R:IR binding ratio, but also did not have prolonged IR residency. None of the new receptor interaction characteristics examined for detemir and glargine was found to be different from human insulin, consistent with a recent study by Varewijck and colleagues,⁷⁷ based on partial dose-response curves. When cultured with cells (L6-hIR) expressing abundant IR, neither glargine nor detemir evoked an increased proliferative response relative to human insulin (whereas the effect of X10 was increased). When the ligands were applied to cells expressing abundant IGF-1R (human mammary epithelial cells [HMEC]), the proliferative response was increased with glargine (and X10), but not with detemir relative to human insulin. These data therefore suggest that any increased mitogenic effect of insulin glargine is likely to be mediated via IGF-1R (or IR/IGF-IR hybrids).

Although insulin glargine shows an increased affinity for IGF-1R and increased mitogenic potential in several *in vitro* models, it must be noted that there are no *in vivo* data to show that this property translates into an increased risk of incident cancer (or an adverse prognosis in established cancer). Furthermore, although well-conducted *in vitro* studies can show up differences between insulin-like ligands in their receptor interaction profiles and in the responses they evoke in isolated cell cultures, the extrapolation of these data into clinical consequence is fraught with difficulties. Clearly, further study is required and better models are needed with which to test insulin analog safety. It has been argued that animal models will potentially provide much better test systems than cell cultures, and ideally these should also be models of human diabetes.⁶⁹ In such systems, the relative effects of study ligands will be mediated and mitigated through

a similar constellation of confounding factors to those seen in patients with diabetes, such as ligand distribution and kinetics, endogenous endocrine factors, adiposity, insulin resistance, etc. The rate of tumor growth in the setting of different insulin (or other) treatments in animals with chemically induced, implanted allograft, or xenograft tumors could be informative. The *in vivo* influence of ligands on transgenic tissue over-expressing receptors such as IGF-1R is another possibility. Such models will potentially enable clinically relevant data to be generated within practical timescales.

Discussion Points Arising

One appealing aspect of diabetic animals as test models is that they are less likely to be vulnerable to hypoglycemia, which has prevented the rodent toxicology study that showed an increase in cancer risk with X10 from being repeated for long-acting insulins. It is important that such models should be able to show a difference between no administration of a ligand, human insulin, and X10.

Concerning whether hyperglycemia would create a more representative environment in an animal model, it was noted that positron emission tomography scanning shows some cancer cells to be extremely efficient at loading glucose independently of insulin, with hypoxia being a powerful stimulus. This was not true of all cancer cells, however, with glucose uptake only detected for some when insulin is given.

MEETING CONCLUSION AND CONCLUDING DISCUSSIONS

Stephen Gough

Despite there being a growing knowledge base that should inform future discussions and research directions, current gaps in our

understanding and different viewpoints about how to interpret available data do not allow a consensus to be reached on how to advise the use of diabetes treatment options, or indeed on how best (or whether) to discuss the issue with patients to reach an 'informed choice.' The subject of how diabetes and its treatments affect cancer is complicated, and the numerous factors involved in the complex signaling networks influencing mitogenic responses, are difficult to tease apart. Putative new animal models are likely to be valuable and informative tools that will help to bridge the gap from laboratory work to clinical predictions. Although some such models have been proposed, including induced tumor, transplanted tumor, and transgenic models (IGF-1R over-expression in breast tissue), these have yet to be developed into fully mature systems.

Further clinical data, however, are also required, hence there remains a need to study the responses of human tumors to different growth factor ligands, and to study the effects of ligands on biomarkers of neoplasm in humans. Epidemiologic study of patients with diabetes and family histories of cancer or precancerous lesions (eg, colonic polyps) could also provide important data, and there is a need to identify any genetic predispositions to cancer that might affect therapy choices. There might also be a 'surgical window' that could be exploited to study the influence of diabetes therapies: the growth of benign tumors could be studied in patients with diabetes awaiting surgical removal of their tumor. As the American Diabetes Association is insisting on the capture of cardiovascular outcome data for new diabetes therapies, an opportunity also exists to routinely capture cancer data in new clinical studies.

Finally, the insulin/IGF axis and its interplay with physiological states in both patients with

diabetes and those individuals with other morbidities span many disciplines. It is therefore clear that greater cooperation and communication between diabetologists, epidemiologists, oncologists, and insulin engineers will be essential if we are to fully understand how peptide hormones can affect cancer risk. Such an understanding should facilitate the engineering of future treatments that are designed to strike the most optimal balance between therapeutic efficacy and molecular safety.

ACKNOWLEDGMENTS

The meeting was convened and sponsored by Novo Nordisk who invited the named authors to speak on invited topics. The sponsor did not influence the content of these presentations beyond their title. All of the authors received honoraria for their presentations. In addition, Stephen Gough is an advisory board member and receives speaker fees from Novo Nordisk, Eli Lilly & Co, sanofi-aventis, GSK and Takeda. Professor Gough also receives research support from Novo Nordisk, Eli Lilly & Co, and Takeda. Christopher Poole has undertaken contract research for Novo Nordisk, Eli Lilly & Co, and sanofi-aventis. Matthias Weber is an advisory board member and has received speaker honoraria from Novo Nordisk. David Russell-Jones has received advisory board fees, lecture honoraria, and research grants from Eli Lilly & Co, Takeda, Pfizer, GlaxoSmithKline, Novo Nordisk, and sanofi-aventis. Bo Falck Hansen is an employee of Novo Nordisk and holds shares in the company. Edoardo Mannucci has received speaking/consulting fees and research grants from Eli Lilly & Co, Novo Nordisk and sanofi-aventis. Jaakko Tuomilehto has received research grants, served as a consultant to/been an advisory board member and given lectures for AstraZeneca, Bayer Pharma, Boehringer

Ingelheim, Eli Lilly & Co, ImpetoMedical, Merck, MSD, sanofi-aventis, Schering-Plough, Pfizer, Novartis, Novo Nordisk, Takeda, and Unilever. He owns shares with Orion Pharma.

All authors had full input at every stage of the drafting of this manuscript, and they would like to thank Murray Edmunds, Michael Pogson, and Natalie Brooks (Watermeadow Medical, Witney, UK) for their writing and editorial assistance in the development of this publication. Support for this assistance was provided by Novo Nordisk. Stephen Gough is the guarantor for this article, and takes responsibility for the integrity of the work as a whole.

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