Supplemental Methods

Fixed cohort analysis

An additional analysis performed was the fixed cohort analysis in which the first exposure to insulin determined in which drug category the participant was categorized. If individuals received additional different types of insulin, switched or discontinued use during follow-up this did not change the exposure status. This was chosen to simulate an intention-to-treat analysis and consequently further avoid reverse causation.

A theoretical example: a participant used six months of insulin glargine, discontinues and starts with another insulin analogue for six months until the end of the study. In the fixed analysis, only the exposure to insulin glargine will be taken into account. In the as treated analysis, the exposure to insulin glargine as well as the exposure to the other insulin analogue will be taken into account. Follow-up for both situations is from the date of starting the first insulin until the end of study. As a consequence of the example described above, the number of exposed participants to a certain insulin will be similar or larger in the as treated analysis in comparison with the fixed analysis. Consequently, the number of participants with a cancer diagnosis will be similar or larger in the as treated analysis.

Propensity Score Analysis

To further adjust for residual confounding, an analysis using propensity scores was performed. The propensity of treatment with either insulin glargine or other insulin analogues at baseline was calculated, based on adjusted estimates from a binary logistic regression model (treatment of interest yes/no) with the following characteristics: sex, age at first insulin prescription, year of first prescription of insulin, number of unique other drugs used in the year before start of insulin (excluding those prescribed for diabetes), number of hospitalisations in the year before start of insulin, the number of days of use of an oral glucose

lowering drug in the year before start of insulin therapy, the number of days of OGLD use as of January 1, 1998. [1-2] The association between respectively insulin glargine and other insulin analogues and cancer in comparison with human insulin was analysed using Cox proportional hazard models with cumulative duration of drug use as a time-varying determinant while adjusting for the respective propensities. Modelling was performed for the fixed analysis as well as for the as treated analysis. In the as treated analysis, adjustments were also made for the use of other insulin than the reference group (human insulin) or the insulin of interest (in the analysis for insulin glargine, adjustments were made for the use of other insulin analogues than insulin glargine and vice versa).

Use of OGLD

Use of OGLD was taken into account in two different ways. Firstly, the full model was stratified for those using less or more than 1 year OGLD prior start of insulin. These models were adjusted for the number of days of use of OGLD as a proxy for duration of diabetes mellitus. Furthermore, the full model was additionally analysed while adjusting in a time varying manner for cumulative use of biguanides (A10BA), sulfonylurea derivatives (A10BB) and use of other OGLD (A10B minus those mentioned above).

Dose

The average dose per insulin category was used as a time-dependent covariable in the full model. However, since follow-up time is used performing these analyses which is methodologically less elegant, a second analysis was performed in which the crude model as well as the full model were analysed stratified for the dose of the first dispensed insulin prescription. The latter being less elegant from a clinical point of view since most participants get initiated on a general dose before being titrated to a more personal dose.

Third, a dose analysis was performed within each insulin category in which the average DDD during follow-up in those with cancer was compared with the average DDD in all individuals without cancer with the same duration of insulin exposure in days. In these analyses, those with an average DDD higher than the median were compared with those with an average DDD lower than the median.

General statistical methods

Covariables that changed the hazard ratio (HR) of cancer risk by more than 10% were considered as confounders. [3] To test for effect modification by covariables mentioned above, interaction terms were introduced in the model and separate analyses were performed if the interaction term was significant. Non-parametric tests (Kruskal-Wallis) and linear regression were applied to verify differences between the treatment groups for continuous variables. These were preferred over ANOVA since there was no equality of variance among the different treatment groups. Differences in categorical variables between the groups were tested with a chi-square test. Analyses were performed using SPSS software (version 16.0, Chicago, US) and SAS software (version 9.1.3, Cary, US). Proportionality of the full model was tested by adding an interaction term of the determinant and time. P-values are two-sided and were considered statistically significant if $p < 0.05$.

Supplemental Results

Fixed cohort analysis

878 participants were hospitalised for cancer, 101 of these started insulin therapy on insulin glargine, 251 started on other insulin analogues and 526 participants started on human insulin. The corresponding incidence rates were respectively 12.12, 12.81 and 14.61 cancers per 1000 patient years. As can be seen from electronic **supplementary material [ESM] Table 3**, use of insulin glargine was associated with a lower risk of malignancies in comparison with users of human insulin (HR 0.73, 95% confidence interval (CI): 0.69, 0.77). In the full model, adjustments were made for age at first insulin prescription, sex, calendar time, number of unique drugs used and number of hospitalisations in the year before start of insulin (HR 0.73, 95% CI 0.73, 0.82). Stratifying for prior OGLD use for less or longer than 1 year did not change this point estimate nor did adjustment for prior days of OGLD used change the point estimates more than 10%. Adjustments were made by adding dose as an additional time-varying covariable to the model (HR 0.79, 95% CI 0.72, 0.82) but since follow-up information is used applying this method, stratified analyses for baseline dose are presented in **ESM Table 3**. Since the vast majority of the cohort members had a median first dose of 16.7 U per day (**Table 1**) these analyses were stratified in three strata: more than, less than or equal to the median dose per day. When replacing cumulative exposure at end of follow up with attained cumulative exposure one year prior to end of follow-up (in order to minimize the chance of reverse causation) the point estimates remained statistically significantly protective. Proportionality of the full model was tested; the assumption of proportional hazards was complied with (p-values respectively 0.14 and 0.67).

When specific cancers were used as endpoints (**ESM Table 4**) applying the full model, insulin glargine was associated with a significantly lower risk of colon cancer but not of other cancers. In contrast, use of insulin glargine was associated with an increased risk of

breast cancer in comparison with human insulin (HR 1.39, 95% CI 1.08, 1.79). The complete analyses for endometrial cancer and pancreas cancer were not possible due to a low number of cancer diagnoses (respectively n=2 and n=7). Furthermore, with regard to the stratified model for first prescribed dose, analyses were not possible for some of the lowest quartiles due to a low number of cases. The low number was a consequence of the issue that ≈70% of the participants received a first dose of 16.6 U per day resulting in an unequal distribution (**table 1**). No clear dose effect could be seen over the different strata of dose. For other insulin analogues, no increased risk of breast cancer was seen (HR 1.00, 95% CI 0.93, 1.09), however, a decreased risk of colon cancer, bladder cancer, respiratory tract cancer and prostate cancer was found.

Dose response relations could not be identified for users of insulin glargine (crude HR comparing those with an average DDD higher than the median with those having an average DDD lower than the median: 1.14, 95% CI 0.77, 1.69, HR applying full model 1.06, 95% CI 0.71, 1.29) , nor could this be demonstrated for other insulin analogues than insulin glargine (crude HR 1.06, 95% CI 0.79, 1.42, HR applying full model 1.04, 95% CI 0.78, 1.39) or for human insulin (crude HR 1.01, 95% CI 0.86, 1.20), adjusted HR applying a comparable full model HR 0.94, 95% CI 0.79, 1.12).

Propensity Score Analysis

In the fixed analysis, the use of insulin glargine was associated with a lower risk of malignancies in comparison with users of human insulin (HR 0.73, 95% CI 0.69, 0.77). The use of other insulin analogues was as well associated with a lower risk of malignancies in comparison with users of human insulin (HR 0.80, 95% CI 0.78, 0.83). Similar estimates were found for the as treated analysis. The use of insulin glargine was associated with a lower risk of malignancies in comparison with users of human insulin (HR 0.72, 95% CI 0.68, 0.76) as

was the use of other insulin analogues in comparison with use of human insulin (HR 0.82, 95% CI 0.79, 0.86).

Supplemental References

[1] Brookhart MA, Schneeweiss S, Rothman KJ, Glynn RJ, Avorn J, Sturmer T (2006) Variable selection for propensity score models. Am J Epidemiol 163: 1149-1156

[2] Rosenbaum P, Rubin D (1983) The Central Role of the Propensity Score in Observational Studies for Causal Effects. Biometrika 79: 516-524

[3] Vandenbroucke JP, von Elm E, Altman DG, et al. (2007) Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Annals of internal medicine 147: W163-194