Breast cancer risk among Swedish hemangioma patients and possible consequences of radiation-induced genomic instability

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Abstract

Breast cancer incidence among 17,158 female Swedish hemangioma patients was analyzed with empirical excess relative risk models and with a biologically based model of carcinogenesis. The patients were treated in infancy mainly by external application of radium-226. The mean and median absorbed doses to the breast were 0.29 and 0.04 Gy, and a total of 678 breast cancer cases have been observed. Both models agree very well in the risk estimates with an excess relative risk and excess absolute risk at the age of 50 years, about the mean age of breast cancer incidence, of 0.25 Gy^{-1} (95% CI 0.14; 0.37) and 30.7 $(10^5 \text{ BYR Gy})^{-1}$ (95% CI 16.9; 42.8), respectively. Models incorporating effects of radiation-induced genomic instability were developed and applied to the hemangioma cohort. The biologically-based description of the radiation risk was significantly improved with a model of genomic instability at an early stage of carcinogenesis.

Key words: Breast cancer risk, models of carcinogenesis, radiation-induced genomic instability

1. Introduction

Starting from the 1920s, in Sweden a large number of individuals were treated for skin hemangioma mainly by external application of radium-226. Since skin hemangioma appear at early infancy the treatment started before the age of two years. Now, about 50 years after the end of treatment, an increase in cancer risk has been observed. In this work the combined skin hemangioma cohorts from Stockholm and Gothenburg are analyzed for female breast cancer incidence since breast cancer is one of most frequent cancer sites for women and an important cause of cancer mortality. Radiation exposure is a well known risk factor for breast cancer; a review of current evidence can e.g. be found in [1]. In earlier analyses of the hemangioma cohort [2, 3, 4], a low but significant excess risk for breast cancer was found. This analysis includes 7 years more follow-up time and almost 2 times as many breast cancer cases.

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The analysis is performed with both the two-stage clonal expansion (TSCE) [5, 6] and an empirical excess relative risk (ERR) model. The TSCE model assumes that the key processes necessary to convert a healthy cell to a cancer cell can be reduced to two basic steps. In spite of this drastic simplification, the model has been applied successfully to various radioepidemiological data sets [7, 8, 9]. Since the TSCE and ERR models are based on very different descriptions of the baseline as well as the radiation risk, a comparison of the risk estimates indicates which characteristics of the risk are inherent in the data and which depend on the choice of model. Since models of carcinogenesis are based on biological transition rates, it is possible to investigate whether biological mechanisms like e.g. genomic instability, bystander effects or low dose hypersensitivity could be seen in cohort data sets [10, 11, 12].

Radiation-induced genomic instability is defined by the occurrence of damages in the progeny of cells which have been exposed to ionizing radiation without expressing any observable effects [13, 14, 15]. Observed damages in the daughter cells include non-clonal chromosome aberrations, increased mutation rates and delayed cell death. Genomic instability induced *in vivo* has been observed *in vitro*: cells have been extracted from the mammary gland of mice at different times after radiation exposure to low-LET radiation [16]. A dose as low as 100 mGy caused delayed chromosomal aberrations. Instability induced *in vitro* can also be transmitted *in vivo* following transplantation of irradiated cells into recipient animals: long-term persistent chromosomal instability was observed in female CBA/H mice after transplantation of exposed bone marrow cells [17]. On the other hand, however, there are conflicting results concerning the occurrence of induced genomic instability in exposed mice or humans [18]. Ongoing research aims at a better understanding of underlying biological mechanisms, e.g. [19, 20, 21].

It is a key question whether radiation-induced genomic instability plays a role in radiation-induced carcinogenesis in humans. In a first analysis it was found that a model with radiation-induced genomic instability described the radiation risk in the Techa River cohort equally well as a model with an age dependent radiosensitivity [22]. Due to the exposure early in life, the Swedish skin hemangioma cohort provides a very good testing ground for models of genomic instability.

The purpose of this study is twofold: the hemangioma cohort is an important cohort for the assessment of breast cancer risk after early exposure and in this study we present an update for the radiation risk, based on a longer follow-up and substantially more cases. Both statistical and model uncertainties are considered and the dependence of risk on attained age is investigated. Second, the hemangioma cohort is used as concrete example to develop models of radiation-induced genomic instability and investigate their possible consequences for the radiation risk.

2. Materials and Methods

2.1. The Study Cohort

In Sweden a large number of individuals were treated with ionizing radiation for skin hemangioma in childhood during 1920 to 1965. Two cohorts, one in Stockholm and one in Gothenburg, with more than 25,000 individuals have been established to study late health effects after radiation therapy in infancy. These cohorts have been described in detail elsewhere [23, 24]. Two thirds of the treated children were females - 9,675 females

in Stockholm and 7,527 in Gothenburg - and in this work we study the breast cancer risk of these women. All persons had their first treatment before the age of 18 months. The number of treatments varied between 1 and 37 with a mean of 1.5 treatments. The establishment and analysis of the hemangioma cohort have been conducted with the permission of the Swedish Data Inspection board, which is responsible for protection of the privacy of the individuals in the database, and the Swedish Ministry of Justice.

2.2. Treatment techniques and dosimetry

In the two cohorts the same treatment techniques were used, mainly with radium-226 applicators [23, 24]. During the first decades it was common to use flat applicators with thin filtration. They were used as β irradiators. During the 1930s, radium-226 needles and tubes became available. They were more heavily filtrated so that all primary α and β particles were absorbed and only the γ rays penetrated. The needles and tubes were usually put into specially made glass or Perspex capsules in order to obtain a fixed distance both between the needles/tubes (4.5 mm and 5.0 mm respectively) and to the underlaying skin (4.5 mm). These capsules were arranged in one or two rows to cover the hemangioma. This treatment method remained the most frequent during the whole study period. The aim of the treatments was to give the hemangioma an average dose of 800 R at 1-10 mm depth, which corresponds to an absorbed dose of 7-8 Gy.

In the Gothenburg cohort the treatments were almost exclusively given with radium-226. For 81% of the treatments needles or tubes were used and in 17% flat applicators. In the Stockholm cohort 65% of the treatments were performed with radium-226 needles or tubes and in 17% flat applicators were used. X-ray treatments, mainly contact therapy ($\leq$60 kVp), were used in the remaining 18%.

The method and procedure for the estimation of the cumulative absorbed dose in different risk organs was done in a similar way for both cohorts. The location of the hemangiomas was coded in 28 different treatment areas over the surface of the body. Dose rates in various risk organs were measured with thermoluminescent dosimeters in a phantom simulating a six month old child. The measurements were complemented and controlled by using a computed dose-planning system. Corrections for two age groups, 0-4 months and 12-17 months, were made to take the differences in body size into consideration. The absorbed dose was estimated for each breast separately 5 mm under the breast nipple. The mean and median absorbed doses of the breasts were 0.29 Gy and 0.04 Gy with a range between 0 and 35.8 Gy.

2.3. Record Linkages

The cohorts were matched by record linkage with several national population registers using the unique identification number given to all Swedish residents. Information on breast cancers diagnosed from 1958 through 2004 were obtained from the Swedish Cancer Register. Using the Swedish Total Population Register, the Emigration Register and the Swedish Cause of Death Register, the follow-up status and person years at risk were estimated. Date of birth and number of children for the women in the cohorts were collected from the Swedish Multi-Generation Register. For 17,158 women (99% of the total) a complete follow-up including reproductive history was available; of these, 15,072 women are still alive and are included in the current follow-up. Six hundred seventy-eight women have been diagnosed with breast cancer. Thirty-one women developed a second

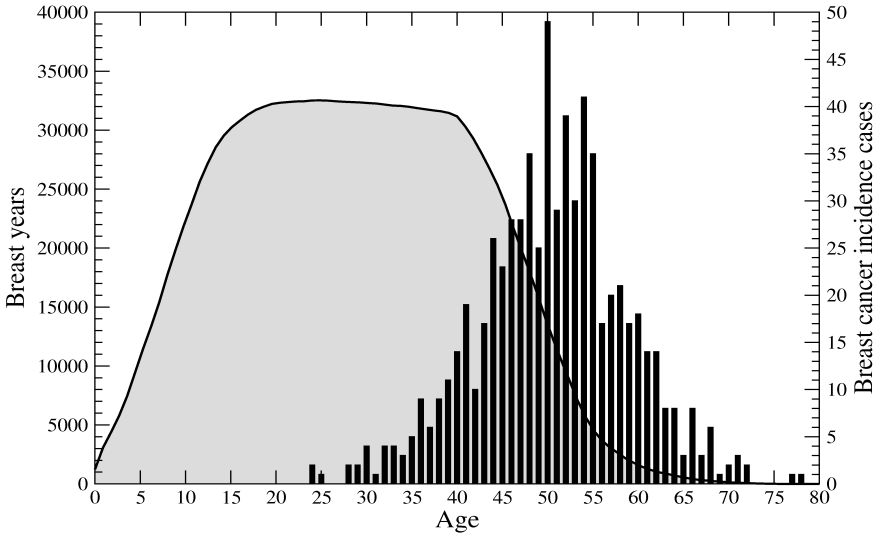

Figure 1: Distribution of breast years and cancer incidence cases with age.

breast cancer, but only the first cancer was included in the analysis. The cohorts have a total of 1,515,308 breast years at risk, calculated from 1958 or date of first treatment to date of first breast cancer, date of death, date of emigration or December, 31,2004. Figure 1 shows the distribution of breast years and cancer incidence cases with age.

2.4. TSCE model for carcinogenesis

In the TSCE model (Fig. 2) it is assumed that the complex process leading to cancer can be reduced to two basic steps. In the first step, called initiation, a healthy cell may experience several genetic or epigenetic events that will result in an intermediate cell. This process occurs with an effective initiation rate $\nu(a)$ where a is the age of the person. The intermediate cells divide with rate $\alpha(a)$ and differentiate or are inactivated at rate $\beta(a)$. A primary intermediate cell together with its daughter cells forms a clone of intermediate cells. The process of clonal growth of intermediate cells is called promotion. In a second step, these intermediate cells convert with the transformation rate $\mu(a)$ to malignant cells. Once a malignant cell is produced, it is assumed to lead to breast cancer, which is diagnosed after a given lag time t_{lag}. We have tested different lag times, but the lag time was found not to have a major influence on the results. So we have chosen $t_{lag} = 5$ years [25] and this value will be used in the rest of this work. The TSCE model is an 'effective' model, i.e., it does not represent a particular biological pathway to breast cancer, but rather includes a multitude of possible cellular processes in its effective parameters that characterize the time scales of an initiation process, clonal growth, and transformation to cancer.

To describe the spontaneous cancer incidence risk, i.e., the baseline hazard, we use constant values of the parameters ν, α, β and μ over lifetime. However, since from epidemiological data only the hazard can be extracted - and not, e.g., the size and distribution of intermediate clones - only three parameters are relevant and one parameter

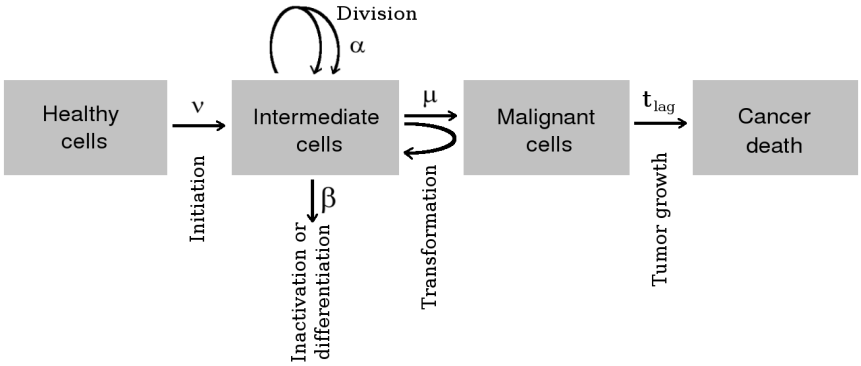

Figure 2: TSCE model

can be chosen freely [26]. We notice that the stem cells of the mammary gland show a monthly cycle [27]. Therefore we fix the division rate to $\alpha = 12.0\ \mathrm{yr}^{-1}$ and keep this value throughout this work. Though this might be a crude approximation, the hazard and all risk estimates are independent of this value. Of course, the values of the other parameters ν, β and μ will depend on this assumption. It is important to note that even for constant parameter values the model predicts an increasing hazard function with age: the number and size of intermediate clones grows with time and thus the probability of a malignant transformation will increase. In the results section the consequences of a change in α will be investigated. It is demonstrated how additional information about the clone size could help to identify the order of magnitude of all parameter values and in this way obtain an indication of effective cellular time scales in the process of cancer development.

To find a good description of the baseline risk, i.e. the cancer incidence risk in the absence of radiation, we have tested the data for possible baseline modification factors. As will be shown in the results section, three factors show an influence on the baseline rates: the number of children, age at first childbirth and screening which was introduced in Sweden after 1985 for women aged 50 or over. In the TSCE model only the first factor - the number of children - gives a significant improvement of the baseline description. Though the screening could be expected to modify the baseline rates, it affects only a small part of the breast years and turns out to be not statistically significant. Thus the baseline parameters are given by

$$\begin{aligned} \nu_{base}(a) &= \nu_0 \\ \alpha_{base}(a) &= \alpha_0 = 12.0\ \mathrm{yr}^{-1} \\ \beta_{base}(a) &= \beta_0\,(1 + f_{ch} \cdot n_{ch}(a)) \\ \mu_{base}(a) &= \mu_0\,, \end{aligned} \tag{1}$$

where n_{ch} is the number of children after some age a, and f_{ch} is a free parameter. So in total there are 4 baseline parameters to be determined from the data. In fact only the combination $N \cdot \nu$ can be calculated where N is the number of stem cells.

The effects of radiation exposure can be incorporated in the model by allowing for a change of the parameters with the dose rate. Since the TSCE model is defined by transition rates, e.g. as the number of transitions per year, the 'direct' effect of radiation is usually implemented by the dose rate (e.g. Gy per year) and not by the accumulated dose: it is assumed that during radiation exposure the cellular changes increase the probability for a transition towards cancer and that this probability increases with the dose rate. For this direct effect of radiation the transition rates are supposed to return to their normal values when the radiation is not longer present. Since the exposure happened in the first two years of life, only an action on the first step - the initiation - can be relevant, since at such an early age almost no initiated clones are present. Among the models in which the initiation rate depends on the actual dose rate, the cancer risk is described best by a linear dependence:

$$\nu(a) = \nu_{base}(1 + r_\nu \cdot d(a)) \,, \tag{2}$$

with $d(a)$ representing the breast dose rate of a specific person at age a and r_ν gives the strength of the radiation action. The dose rate is very large during the treatment times and zero in the remaining time.

For piecewise constant parameters the TSCE model can be solved stepwise analytically [28] and the hazard $h(a)$ can be determined. Since the dose is known for each breast separately, the total likelihood L_{tot} can be obtained from the product of the individual likelihood of each breast for all cohort members $L_{tot} = \prod_{i,b} L_{i,b}(\Psi_{i,b}; a_{1i}, a_{2i})$ [8], where b is an indicator for the left or right breast and $\Psi_{i,b}$ is the survival function for breast b of person i, and a_{1i} and a_{2i} are the ages at beginning and end of follow-up. This method does not group data, but takes into account the individual exposure history of each person. To determine the best values of the parameters we have performed a maximum likelihood (minimum deviance) fit of all parameters simultaneously using the program MINUIT from the CERN library [29]. The best fit of the parameters is obtained by minimizing the deviance

$$\mathrm{Dev} = -2 \, \ln L_{tot} \,. \tag{3}$$

Once the parameters have been obtained, the excess relative risk per unit dose (ERR_{pd}) and excess absolute risk per unit dose (EAR_{pd}) for each person i at age a can be computed by

$$\begin{aligned} \mathrm{ERR}_{pd,i}(a) &= \left(h_i(a)/h_{base,i}(a) - 1\right)/D_i(a - t_{lag}) \\ \mathrm{EAR}_{pd,i}(a) &= \left(h_i(a) - h_{base,i}(a)\right)/D_i(a - t_{lag}) \,, \end{aligned} \tag{4}$$

where $D_i(a - t_{lag})$ is the total accumulated dose at $a - t_{lag}$; the hazard $h_i(a)$ depends on the exposure history of person i and thus can be different for two persons with the same age and the same accumulated dose. Also models with a linear-quadratic dose dependence and threshold models where the initiation rate is increased only if the applied dose surpasses a certain threshold value are investigated. The $\mathrm{ERR}_{pd}(a)$ and $\mathrm{EAR}_{pd}(a)$ for the total cohort at a certain age a can then be obtained by averaging over the persons at risk.

For an estimate of the uncertainty bounds we have simulated 10,000 Monte Carlo realizations from the parameter distributions. Since the uncertainties of the parameters turn out to be distributed almost symmetrically we have assumed a usual Gauss distribution. In a computer program written by one of us (M.E.) we have created these

realizations taking into account the correlation matrix of the parameters given by MINUIT within a distribution-free approach [30] and using Latin Hypercube Sampling [31]. We have checked the program for the correct distributions and correlations and compared to the results from Crystal Ball [32] as a double check. For each realization the baseline hazard $h_{base}(a)$, ERR_{pd}(a) and EAR_{pd}(a) of the cohort can be calculated for each age a. The values of the percentiles of the full set of realizations then provide the uncertainty bounds.

2.5. Models of genomic instability

Radiation-induced genomic instability (GI) [13] describes an increased rate of new alterations in the genome after exposure to ionizing radiation. It is observed in the progeny of cells many generations after the initial exposure, a review on the evidence of radiation-induced genomic instability *in vitro* and *in vivo* can be found in [14, 15]. In principle, GI could appear at any stage in the carcinogenic process and the question whether GI is an early event is a major question of cancer genetics [33].

Since the TSCE model is described by biological parameters it is possible to investigate whether potential consequences of GI are expressed in the data. We assume that the appearance of radiation-induced GI will increase the effective rates of initiation, promotion or malignant conversion, and this increase will take place not only during the radiation exposure, but also at later times. Many variations are possible, e.g., mutations could show up directly after exposure or with a certain time lag, doses received more recently could have a stronger effect than doses received longer time ago, or the mutations may need an activation dose to appear. The outcomes of such a model testing should be interpreted with caution since positive or negative results do not (dis)prove GI, but could only indicate if the data are consistent or not with the GI hypothesis.

We have analyzed different variations of the TSCE model by using a standard radiation action on initiation as in Eq. (2) and then incorporating the above mentioned effects. Some of the above mentioned models are discussed in ref. [22]. In our case the description of the radiation risk is significantly improved if after exposure the initiation rate is increased during the whole lifetime proportional to the total accumulated dose, $D(a)$, until age a:

$$\nu(a) = \nu_{base}\,(1 + r_\nu \cdot d(a) + r_{\mathrm{GI}} \cdot D(a))\ . \tag{5}$$

Also models with a genomic instability in promotion or the malignant conversion rate μ were tested, but no significant improvement was found. To illustrate the fundamental difference between direct TSCE models, where a direct effect changes the transition rates only during the radiation action as in Eq. (2), and models including effects of radiation-induced genomic instability, Fig. 3 shows the change of the initiation rate with age after an exposure of 1 Gy in the age period of 12 to 18 months. For the simple initiation model of Eq. (2) the initiation rate is strongly increased during the action of radiation and reduces to it spontaneous value afterwards. In the model with GI of Eq. (5) the immediate increase is smaller, but afterwards a permanent enhancement remains: due to the cellular change it is more likely that in the future intermediates cells will be produced.

2.6. Excess relative risk model

We use general parametric models to describe the hazard function in the excess relative risk model. It turns out that both the number of children n_{ch} and the age at first

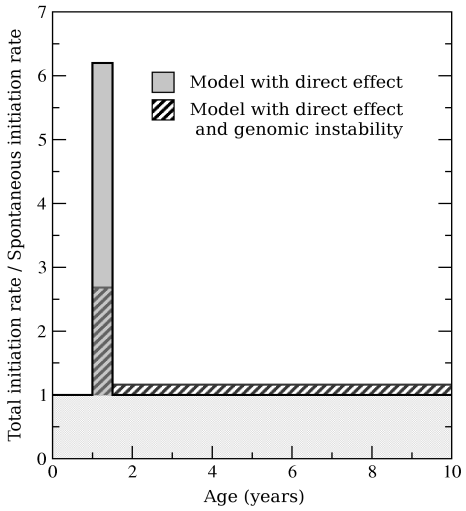

Figure 3: Change of the initiation rate for the TSCE models of Eqs. (2) and (5) after an exposure of 1 Gy in the age period of 12 to 18 months.

childbirth a_{1cb} are significant baseline corrections, as will be demonstrated in the results section. Therefore the hazard is given by

$$\begin{aligned} h(a) &= 10^{-5} \cdot e^{\psi(a)} \cdot (1 + \mathrm{ERR}_{pd}(a) \cdot D(a - t_{lag})) \\ \psi(a) &= \psi_0 + \psi_1 \cdot \ln \frac{a}{50} + \psi_2 \cdot \ln^2 \frac{a}{50} \\ &\quad + f_{ch} \cdot n_{ch}(a) + f_{1cb} \cdot (a_{1cb} - 30) \cdot \theta(a - a_{1cb}) \,, \end{aligned} \tag{6}$$

where $\theta(a - a_{1cb})$ is one when the age is larger than the age at first childbirth and zero otherwise. The ERR model is applied both for a constant ERR_{pd} and a linear risk model where the ERR_{pd} depends linearly on attained age, $\mathrm{ERR}_{pd}(a) = \mathrm{ERR}_{pd}(50) + \mathit{slope} \cdot (a - 50)$. In addition, models with a log-linear dependence on age are investigated as well as models with a linear-quadratic dose response and threshold models that have no risk below a certain threshold value. In principle, since the dose was received in early childhood, but the cancers developed much later in life, the lag time is irrelevant for the risk estimates; however, for consistency we include the lag time for the ERR models as well. The data were analyzed both with individual maximum likelihood methods as was done for the TSCE models and with Poisson regression after stratification with very similar results. For a better comparison to the TSCE models the results from the individual likelihood fit are presented.

3. Results

In the TSCE baseline model of Eq. (1) the number of children turns out to be a significant (95% level) baseline effect: the introduction of the $f_{ch} \cdot n_{ch}$ term to the apoptosis

TSCE model	No. of parameters	Deviance	p-value
Pure baseline (Eq. 1)	4	10601.9	
Simple initiation (Eq. 2)	5	10562.7	$3.8 \cdot 10^{-10}$
Initiation with threshold	5	10564.0	$7.4 \cdot 10^{-10}$
Initiation linear-quadratic in dose	6	10562.7	1.0
Initiation with GI (Eq. 5)	6	10558.1	0.032
ERR model			
Pure baseline	5	10599.3	
Constant ERR	6	10555.8	$4.2 \cdot 10^{-11}$
ERR with threshold	6	10556.4	$5.8 \cdot 10^{-11}$
ERR linear-quadratic in dose	7	10555.2	0.44
ERR linear in age	7	10555.3	0.48
ERR log-linear in age	7	10555.5	0.58

Table 1: Comparison of different TSCE and empirical ERR models, the best models are emphasized. The models with threshold are shown for thresholds of 100 mGy. P-values are given relative to the best model with one parameter less.

rate, and thus modifying promotion, reduces the deviance by 14.3 points (p=0.00016). Introducing an age at first childbirth modifier, in addition to the number of children, the deviance goes down by 0.7 points, which is not significant (p=0.40). However, since both effects are correlated - woman with childbirth at younger ages will in average have more children - it is difficult to disentangle both contributions: if we introduce an age at first childbirth modifier without the number of children effect, the change in deviance of 10.8 points is again significant (p=0.0010) which is nevertheless not as good as the number of children modifier.

In the ERR baseline model both the number of children and the age at first childbirth are significant: the number of children modifier reduces the deviance by 14.3 points (p=0.00016) and the age at first childbirth modifier by additional 5.6 points (p=0.018). In neither model is a significant birthyear effect found: with a birthyear confounder the deviance reduces by 0.5 points in the TSCE model (p=0.48) and by 0.8 points in the ERR model (p=0.37).

To check for a possible screening effect it is assumed that screening would lead to an increased hazard, relative to the hazard without screening, after calendar year 1985-90 and after age 45-55. However, introducing such a multiplier did not give a stable significant improvement of the deviance. The number of breast years and breast cancer cases after introduction of the screening program is probably not large enough to give significant results up to the current end of follow-up. Thus we did not include a screening modifier in our baseline description.

Using the baseline parametrisation as in Eqs. (1,6) in Table 1 we compare different models, including radiation effects, by deviance. The first line of the TSCE model gives the pure baseline model without radiation and the second line shows the simple initiation TSCE model of Eq. (2) including a radiation effect on the initiation. The p-values in Table 1 are given relative to the best model with one parameter less. A model with threshold, i.e., the initiation rate is increased only if the applied dose surpasses a certain threshold value, does not improve the fit as compared to the simple initiation TSCE

model. Indeed, it is seen that for a threshold value of 100 mGy the deviance is even larger. Different threshold values between 0 and 500 mGy give similar results. Thus we neither find support for a threshold model nor can it be ruled out. A model with a linear-quadratic dose response includes an additional term which allows for a possible quadratic dependence of the initiation rate on the dose rate. It turns out that this quadratic contribution is practically zero with very large uncertainties and that the deviance is not measurably reduced. The TSCE model with GI of Eq. (5) reduces the deviance by 4.6 points (p=0.032) and is a significant improvement to the simple initiation TSCE model. We have also tested models where the GI contribution to the initiation rate was less than linear in the accumulated dose, as e.g. observed in [34], but we found no improvement to the linear one.

The first two lines for the ERR model show the baseline model and the ERR model with a constant excess relative risk per unit dose. As for the TSCE model, an ERR model with threshold, i.e., assuming no risk below a threshold of 100 mGy, results in a deviance even larger than for the constant ERR model. In the ERR model with a linear-quadratic dependence in dose an additional parameter with a quadratic dependence on dose is introduced. However, the uncertainty in this parameter is large and the deviance in reduced by 0.6 points which is not significant (p=0.44). The next line shows the ERR model with a linear dependence of risk on attained age. Though the improvement is not significant and we will therefore use the constant ERR model as our main excess relative risk model, in the following we will also compare the risk prediction of this model to the TSCE model. A log-linear model in age gives a very similar dependence of the excess risk with age as the linear model, though the linear model is slightly better. We also checked both for the TSCE and ERR models if the number of treatments or the average dose rate during treatment time might modify the results, but we found no indication for a change in the radiation risk. Comparing the best TSCE and ERR models, i.e. the TSCE model with GI and the constant ERR model, the deviance of the ERR model turns out to be slightly lower by 2.3 points, most probably due to a somewhat better description of the baseline.

In Table 2 the parameters of the TSCE model with GI and of the constant ERR model are given with the 1σ error bounds. From the values $f_{ch} = -0.084$ and $f_{1cb} = 0.022$ of the ERR model, the change in baseline risk translates as follows: for each child the risk is lowered by 8% and the risk is further reduced for a childbirth at young age, e.g., with a first childbirth at 20 years the breast cancer risk is 20% lower than for a first childbirth at age 30. Since the corresponding parameter for the number of children f_{ch} in the TSCE model is positive, each child increases the apoptosis rate and reduces the risk as in the ERR model.

Table 3 shows the predicted and observed distribution of cancer incidence cases by dose category for the TSCE model with GI and the constant ERR model. The predictions of both models are very similar. The models predict about 47 excess cases. In Fig. 4 the hazard is given for both models together with the hazard of observed cases, grouped in intervals of 3 years; the standard deviation of the observed hazard is estimated (assuming a Poisson distribution) from the square root of the number of observed cases.

In Fig. 5 the ERR_{pd} as function of age is shown. In the interval between 35 and 65 years about 90% of all cancer cases occurred and in this age range we expect our models to give a good approximation to the risk. The error bars are shown for the 68% confidence interval instead of the 95% confidence interval - which would be about twice as large -

TSCE parameters	Value	Error
$N \cdot \nu_0$ [yr^{-1}]	0.14	$\pm$0.02
β_0 [yr^{-1}]	11.78	$\pm$0.016
μ_0 [yr^{-1}]	$4.23 \cdot 10^{-7}$	$\pm 1.53 \cdot 10^{-7}$
f_{ch} [1]	0.0011	$\pm$0.0003
r_ν [yr Gy^{-1}]	0.74	$\pm$0.91
$r_{\rm GI}$ [Gy^{-1}]	0.18	$\pm$0.09
ERR parameters		
ψ_0 [1]	5.02	$\pm$0.08
ψ_1 [1]	3.63	$\pm$0.23
ψ_2 [1]	-5.09	$\pm$0.81
f_{ch} [1]	-0.084	$\pm$0.039
f_{1cb} [1]	0.022	$\pm$0.0090
ERR$_{pd}$[Gy^{-1}]	0.240	$\pm$0.055

Table 2: Best fit and 1σ uncertainties of the parameters from the maximum likelihood analysis of the TSCE model with GI of Eqs. (1,5) with $\alpha_0 = 12.0$ yr^{-1} and the constant ERR model of Eq. (6).

Dose [mGy]	Breast years	Baseline prediction		Model prediction		Observed
		TSCE	ERR	TSCE	ERR	
$0-50$	838055	351.9	352.4	353.2	353.7	350
$50-100$	241770	96.8	96.7	98.7	98.5	103
$100-200$	189197	76.2	76.2	79.0	78.9	79
$200-300$	85129	35.7	35.7	37.8	37.8	44
$300-400$	37908	16.5	16.4	17.9	17.8	23
$400-1000$	54398	23.6	23.6	27.2	27.1	17
$1000-4000$	45011	18.5	18.6	26.4	26.3	23
>4000	23840	11.2	11.3	37.8	37.9	39
Total	1515308	630.4	630.9	678	678	678

Table 3: Number of baseline, observed and predicted breast cancer incidence cases based on the TSCE model with GI and the constant ERR model.

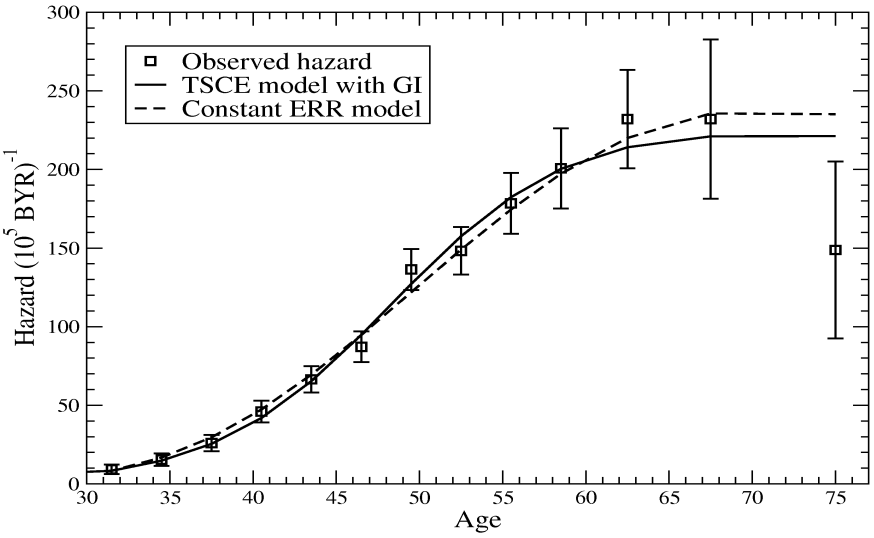

Figure 4: Comparison of the observed hazard with standard deviation to the TSCE model with GI and the constant ERR model.

in order to see more clearly the magnitude of the differences between the models. At the age of 50 years which almost coincides with the average age at breast cancer incidence of the cohort of 50.6 years, for the TSCE model with GI we obtain as central risk estimates:

$$\begin{aligned} \mathrm{ERR}_{pd}(50) &= 0.250\ \mathrm{Gy}^{-1}\ (95\%\ \mathrm{CI}\ 0.14; 0.37) \\ \mathrm{EAR}_{pd}(50) &= 30.7\ (10^5\ \mathrm{BYR\ Gy})^{-1}\ (95\%\ \mathrm{CI}\ 16.9; 42.8)\,, \end{aligned} \tag{7}$$

and all models agree well for these risk predictions. However, looking at the change of the ERR_{pd} with age, the simple initiation TSCE model predicts a strong decrease of ERR_{pd} with age. The TSCE model with GI improves the description of the data significantly and it can be seen that it agrees very well with the predicted excess risk from the ERR model with a linear slope. It is important to note that the TSCE and ERR models are based on very different descriptions of the baseline risk and also the radiation risk is implemented in a different way - depending on the dose rate acting on the initiation in the TSCE model and the total accumulated time-lagged dose in the ERR model. Nevertheless both models find an astonishing agreement not only for the central risk estimate, but also on the change of ERR_{pd} with age in spite of a substantial uncertainty with $slope = -0.0047 \pm 0.007$ (yr Gy)$^{-1}$ (1σ error). In Fig. 5 it can be seen that the ERR_{pd} of the simple initiation TSCE model is outside the 1σ uncertainty bounds for older ages whereas the constant ERR model remains within the error bounds. Indeed the constant ERR model is compatible with the linear slope ERR model, but the simple initiation TSCE model describes the data significantly worse than the TSCE model with GI. Table 4 summarizes the results for the breast cancer risk of the hemangioma cohort; the ERR_{pd} and EAR_{pd} estimates of the TSCE model with GI and the constant ERR model are given for different attained ages together with the 95% confidence interval.

The TSCE model does not represent a particular cellular process, but should rather

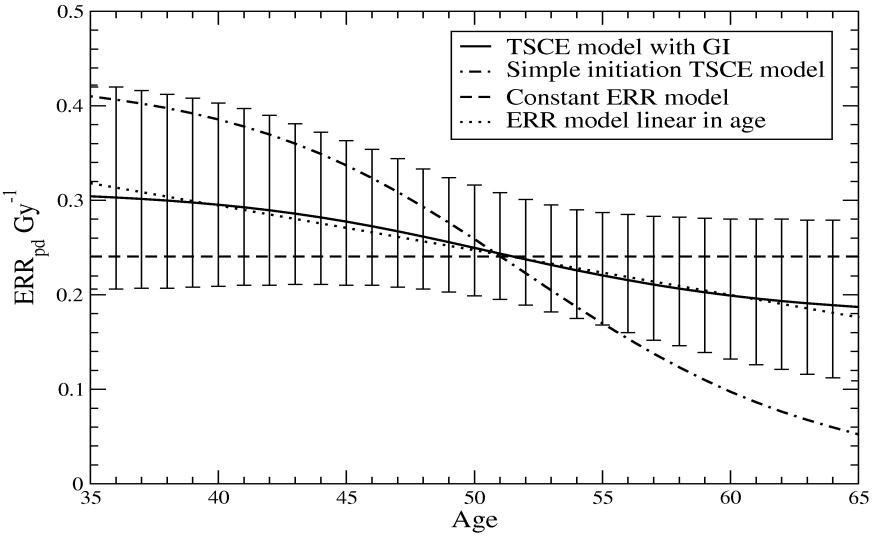

Figure 5: ERR_{pd} for the different models as a function of attained age with the 68% CI. For clarity, the error bars are shown only for the TSCE model with GI, the uncertainties of the other models are of similar size.

	TSCE model with GI	Constant ERR model
ERR_{pd} (40 years)	0.299 (0.14; 0.51)	0.240 (0.13; 0.35)
ERR_{pd} (50 years)	0.250 (0.14; 0.37)	0.240 (0.13; 0.35)
ERR_{pd} (60 years)	0.202 (0.07; 0.36)	0.240 (0.13; 0.35)
EAR_{pd} (40 years)	10.6 (4.3; 16.5)	9.8 (5.6; 14.1)
EAR_{pd} (50 years)	30.7 (16.9; 42.8)	28.4 (16.3; 40.5)
EAR_{pd} (60 years)	39.0 (11.8; 65.5)	46.1 (26.1; 65.9)

Table 4: Predictions of the ERR_{pd} and EAR_{pd} with 95% CI of the TSCE model with GI and the constant ERR model for different ages . The ERR_{pd} (EAR_{pd}) values are shown in units of Gy^{-1} and (10^5 BYR $\mathrm{Gy})^{-1}$ respectively.

be seen as effective model that includes a multitude of pathways. Thus the knowledge of the biological parameters could give an indication of effective cellular time scales leading to cancer. However, since one parameter in the TSCE model can be chosen freely, cohort data alone are not sufficient to determine the order of magnitude of the biological parameters. Now we demonstrate how additional information about the clone size could help to obtain an estimate for the size of the parameters. The calculation of the TSCE models have been performed with a fixed division rate $\alpha_0 = 12.0$ yr^{-1}. We investigate the predictions of the model for largely different values of α_0. In Table 5 three different parameters sets for $\alpha_0 = 1.0$, 12.0 and 50.0 yr^{-1} are shown. As already mentioned, the deviance, hazard and ERR$_{pd}$(EAR$_{pd}$) of all parameter sets are exactly equal. Nevertheless the biological transition rates change, e.g. in the model with $\alpha_0 = 1.0$ yr^{-1} intermediate clones are produced with a much lower frequency of $N \cdot \nu_0 = 0.011$ yr^{-1} than in the model with $\alpha_0 = 50.0$ yr^{-1} where $N \cdot \nu_0$ is 0.571 yr^{-1}; the transformation rates, on the other hand, show the inverse behavior. The parameter for the number of children changes in such a way that the change in the apoptosis rate $\Delta\beta = \beta_0 \cdot f_{ch}$ remains the same. Since the radiation acts exclusively on the initiation, the risk is directly proportional to the change in the initiation rate and consequently the parameters r_ν and r_{GI} are the same in all parameter sets. With Monte Carlo methods we have simulated the creation and growth of the intermediates clones after an exposure of 1 Gy at the age of one year.

The models differ significantly in the number of cells in the active clones, where an active clone neither has died (zero size) nor has become malignant. The number of active clones, however, is similar in the different models. The larger α_0, the more clones are produced in the first instance. At age 45, with a time lag of 5 years corresponding to a hazard at age 50, for $\alpha_0 = 50.0$ yr^{-1} an average of 30.7 clones per persons are produced. But a large number (99.3%) of the clones die, i.e. reach size zero before they can grow further or turn malignant, and only 0.197 active clones per person remain. For $\alpha_0 = 1.0$ yr^{-1} only 0.59 clones are produced, but the probability of clone death is smaller with 74.4% and 0.136 active clones remain in average. A very significant distinction between the parameters sets is given by the different size of the active clones: whereas for $\alpha_0 = 1.0$ yr^{-1} the average clone size is about 2,920 cells, but with a relatively large probability to turn malignant, the clone size for $\alpha_0 = 50.0$ yr^{-1} is much larger with 106,800 cells in average, but also the probability for a malignant transformation is smaller. Thus the knowledge of the average clone size would permit to estimate approximate values of the biological transition rates. It can be seen that the increase of risk with age is primarily a result of the growth of existing clones and not due to an increase of the number of active clones which increases only slightly. The numbers presented in Table 5 have been given for an exposure of 1 Gy at the age of 1 year. The exposure increases the production of clones: without exposure the number of active clones would be about 20% smaller. However, the average number of cells in the clones would not change much.

127 women in the cohort received doses of more than 10 Gy to the breast. Since doses above 10 Gy are usually not relevant for radiation protection we have re-analyzed the cohort with a dose cutoff at 10 Gy using the same models as before. An obvious change is the predicted number of excess cases of about 30 breast cancer cases, compared to almost 50 excess cases for the full cohort. This reduces the statistical significance substantially. However, the central risk estimate is still significant (95% level) with a central value from

TSCE parameters	Set 1	Set 2	Set 3
α_0 [yr^{-1}]	1.0	12.0	50.0
$N \cdot \nu_0$ [yr^{-1}]	0.011	0.14	0.57
β_0 [yr^{-1}]	0.78	11.78	49.78
μ_0 [yr^{-1}]	$5.08 \cdot 10^{-6}$	$4.23 \cdot 10^{-7}$	$1.02 \cdot 10^{-7}$
f_{ch} [1]	0.017	0.0011	0.00027
r_ν [yr Gy^{-1}]	0.74	0.74	0.74
$r_{\rm GI}$ [Gy^{-1}]	0.18	0.18	0.18
Average number of active clones			
35 years	0.120	0.159	0.180
45 years	0.136	0.176	0.197
55 years	0.141	0.181	0.200
Average number of cells in active clones			
35 years	930	8,960	33,440
45 years	2,920	28,560	106,800
55 years	3,900	38,070	141,800

Table 5: Parameters of the TSCE model with GI of Eqs. (1,5) for values of $\alpha_0 = 1.0, 12.0$ and 50.0 yr^{-1}. The deviance and ERR$_{pd}$(EAR$_{pd}$) estimates are exactly equal for all parameter sets. The number and size of clones are given for an exposure of 1 Gy at the age of 1 year. With a time lag of 5 years, the number and size of clones at age 45 determine the hazard at age 50. The number of clones and clone size is given for active clones defined by neither having died (size zero) nor having become malignant.

the TSCE model with GI of

$$\begin{aligned} \mathrm{ERR}_{pd}^{<10\mathrm{Gy}}(50) &= 0.20\ \mathrm{Gy}^{-1}\ (95\%\ \mathrm{CI}\ 0.077; 0.35) \\ \mathrm{EAR}_{pd}^{<10\mathrm{Gy}}(50) &= 24.6\ (10^5\ \mathrm{BYR\ Gy})^{-1}\ (95\%\ \mathrm{CI}\ 8.7; 39.3)\,, \end{aligned} \tag{8}$$

which is slightly lower than the risk for the full cohort. All models agree very well for this central risk estimate. However, the TSCE model with GI is not a significant improvement to the simple initiation TSCE model any more (p=0.29), the main reason being the reduced statistics which makes an estimate of the slope more uncertain. Also the slope is a bit steeper with $slope = -0.0062 \pm 0.0085$ (yr Gy)$^{-1}$ (1σ error) which further reduces the difference between both TSCE models. Even with the reduced statistical power, the TSCE model with GI and the ERR model with a linear slope - though based on completely different background and risk descriptions - give almost identical results and the graph of the change of ERR$_{pd}$ with age is very similar to Fig. 5.

4. Discussion

The Swedish hemangioma cohort has several remarkable features: a unique property is the very early exposure from birth to the age of 2 years which makes it an ideal cohort for the study of radiation risk after exposure at early childhood. Furthermore it facilitates the development of biologically-inspired models, like the TSCE model with GI, since it strongly limits the number of possible models. It is a large cohort of 17,158 women and a long follow-up time of 50 years. In this study data on breast cancer incidence until

December 31, 2004 was analyzed. The exposure covers a wide range of doses to the breasts up to 36 Gy with a generally good quality of dosimetry. A large part of the cohort of 15,072 (88%) women is still alive and under continuous investigation.

The analysis of ref. [4] included the hemangioma cohort updated to 1997 with 360 breast cancer cases and an ERR_{pd} of 0.34 Gy^{-1} (95% CI 0.20; 0.52). Ref. [3] had a follow-up until 1995 with 307 breast cancer cases, incidence rates were derived from the general Swedish population. In ref. [4] all Swedish women born 1920-1959 were included in the analysis and no external incidence rates were used. In this work the parameters of both the TSCE and ERR models were determined by the cohort without external reference. The direct comparison of the TSCE and the empirical ERR models gives important insights to the model dependence of the results: both models agree well for the hazard as seen in Fig. 4. For the baseline confounders, the models differ in the significance of the age at first childbirth; however, due to the large correlation with the number of children confounder, it is difficult to disentangle both effects. Both models nevertheless agree very well in their risk predictions and a central risk estimate for the cohort is given by the excess risk at the age of 50 years, about the mean age of breast cancer incidence, of $\mathrm{ERR}_{pd}(50)$=0.25 Gy^{-1} (95% CI 0.14; 0.37). This confirms the previous result [2] of $\mathrm{ERR}_{pd}(44)$=0.35 Gy^{-1} (95% CI 0.18; 0.59) for a mean breast cancer incidence age of 44 years. A linear dependence of risk on dose was found with no indication of a quadratic term. Though the change of risk with age has a significant uncertainty, both models indicate a relatively moderate decrease of ERR_{pd} with age. In fact, using an ERR model with a linear dependence of excess risk on age, the TSCE model with GI and the ERR model coincide almost perfectly. The agreement is the more astonishing as the TSCE and ERR models use completely different parameterizations of the baseline and the radiation risk. This is a strong indication that the decrease of ERR_{pd} with age is contained in the data and not a model-specific property.

The Canadian fluoroscopy study [35] included 31,917 women treated for tuberculosis between 1930 and 1952 with 688 breast cancer deaths and a dose range up to 18.4 Sv. A decrease of risk with increasing age at exposure was observed and the total risk for age at exposure of 15 years was determined to ERR_{pd}=0.90 Sv^{-1} (95% CI 0.55; 1.39). The risk was driven upwards by a relatively small subcohort of 2,266 women from Nova Scotia with a risk of ERR_{pd}=3.56 Sv^{-1} (95% CI 1.85; 6.82) whereas the cohort without Nova Scotia had a risk comparable to the hemangioma cohort of ERR_{pd}=0.40 Sv^{-1} (95% CI 0.13; 0.77).

In [36] a pooled analysis of breast cancer risk of several cohorts was performed. Even taking into account the diverse properties of the cohorts, which differed substantially in baseline risk, dose range, exposure pattern or age at exposure, the results for the ERR_{pd} varied widely without an easy explanation for the differences. In the LSS cohort with a high dose-rate exposure a risk of $\mathrm{ERR}_{pd}(50)$=2.10 Gy^{-1} (95% CI 1.6; 2.8) was found. A decrease of ERR_{pd} with attained age was observed, but no additional age at exposure modifier was needed. The same behavior was observed in the Massachusetts fluoroscopy cohort [37], but with a much lower risk of $\mathrm{ERR}_{pd}(50)$=0.74 Gy^{-1} (95% CI 0.4; 1.2). On the other hand, the Swedish benign breast disease cohort [38] had a strong dependence on age at exposure and no significant variability with age attained; for an age at exposure of 25 years the risk was determined to ERR_{pd}=1.9 Gy^{-1} (95% CI 1.3; 2.8). In [36] it has been proposed that exposure with low dose rates as in the hemangioma cohort, e.g. compared to the LSS cohort, might be responsible for the much lower ERR_{pd}, though

in our opinion the dose rate in the hemangioma cohort is not really small. However, in view of the fact that large cohorts such as the Techa River cohort [22] or the 15-country nuclear worker study [39] with very low dose rates and an exposure over several years find a larger ERR_{pd} for all solid tumors than the LSS cohort [40, 41], the low dose rate argument for breast cancer does not seem very convincing.

A special property of the hemangioma cohort is the very early age at exposure before the age of 2 years. In the framework of the TSCE model (or any multistep model) this has the consequence that only a radiation action on the first step, the initiation, can be relevant for the radiation risk: even if promotion would be enhanced by radiation, i.e. an increase of the division rate α or a decrease of the apoptosis rate β by radiation, at such an early age there are almost no intermediate cells present that could be 'promoted'. Furthermore the time pattern of promotion is different: even if a radiation effect on promotion was present, the ERR_{pd} would decrease rapidly after the age of 50, whereas in the data only a small decrease in the excess relative risk is observed. This also holds true if promotion was enhanced permanently like in the GI model of initiation.

Thus the cohort provides an excellent opportunity to test whether a radiation effect on the initiation is present and to study the exact form of the interaction since no mixing with a radiation effect on promotion is possible. The cohort demonstrates clearly that a radiation effect at an early stage of carcinogenesis exists: Table 1 shows that the difference between a pure baseline model and a model with simple initiation amounts to a difference in deviance of 39.2 points ($\mathrm{p}=3.8 \cdot 10^{-10}$). If radiation in a general cohort, i.e. with exposure at all ages, had an effect both on initiation and on promotion or transformation, this might help to explain the particular low risk in the hemangioma cohort: only at ages later in life enough initiated cells are present so that radiation on the promotion step could increase the cell damage and contribute to the radiation risk at older ages.

Though the TSCE model with direct simple initiation agrees very well with the ERR model for the central risk estimate, it predicts a strongly decreasing ERR_{pd} with age. The description of the radiation risk is significantly (95% CI) improved with a model including genomic instability: in the GI model it is assumed that some kind of cell damage remains permanently, enhancing the production of intermediate cells over all lifetime. Although this permanent damage could in principle also act on the promoting or the transformation step, in this cohort a significant GI effect was only seen for the initiation. Compared to the GI model for all solid cancer in the Techa River cohort [22] both models are similar with the difference that in the Techa River cohort the onset of GI was only observed after an exposure to radiation after an age of about 30 years. Due to the early exposure, such a model is not possible for the hemangioma cohort. Furthermore, in the Techa River cohort an equivalent model to GI existed, with different radiosensitivity for younger and older ages. In the hemangioma cohort, however, it is not possible to construct such alternative models and thus the hemangioma cohort presents a very clear testing ground for models including effects of GI. One drawback of the cohort is the fact that a part of the excess cases originates from persons with doses above 10 Gy. With a dose cutoff at 10 Gy the central risk estimate is only slightly lower, but the statistics is not sufficient any more to see a significant effect of GI. The cohort is very promising for the future: most women are still under active follow-up with an age mainly between 50 and 70 years. Whereas now the mean age at breast cancer is about 50 years, more cancers at older ages are expected for the next years. The difference in the risk

predictions of the TSCE models with direct effect and with GI is not very large until an age of about 50 years, but increases strongly afterwards. Thus in the near future the distinction between these models will become much stronger and the evidence for the existence of GI might be put on a firmer basis.

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