Quantitative analysis of tumor growth and response to therapy

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Göteborg 2010

Doctoral Thesis

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Printed in Sweden by:

Chalmers reproservice, Göteborg 2010

ISBN 978-91-628-7997-6

Eprint: http://hdl.handle.net/2077/21548

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Abstract

Modeling the natural growth of tumors is of value for evaluation of tumor progression and optimization of treatment strategies. However, modeling tumor growth based on clinical data is hampered by the limited data available, since therapy is in general initiated as early as possible after diagnosis. Most descriptions of tumor growth rate are thus based on two data points per tumor, and assuming exponential tumor growth. The time needed for a tumor to double in volume, doubling time (DT), is widely used for quantification of tumor growth rate. Growth rate can also be quantified using specific growth rate (SGR), equal to ln2/DT. Some studies have shown non-exponential growth characteristics if tumors are observed for a relatively long period, usually with a reduced relative growth rate with time. Current criteria for evaluation of tumor response to therapy, e.g. RECIST, use change in tumor size as a measure and do not consider the natural tumor growth during observation. Knowledge of the natural growth model would thus provide a better assessment of therapeutic response.

In this study, mathematical analyses and computer simulations were used for theoretical evaluation of parameters for tumor growth, together with evaluation and application to clinical data. DT and SGR were compared for their accuracy as a quantity for tumor growth rate. The relation between growth rate and tumor volume was used for estimation of tumor growth model and tumor dissemination rate. A general model for tumor response to therapy was developed assuming that an effective treatment may decrease the cell proliferation rate (cytostatic effect) and/or increase the cell loss rate (cytotoxic effect) of the tumor.

The results showed that, beside the fact that DT is not defined when two consecutively measured tumor volumes are equal, when DT is used for quantification of tumor growth rate, data is transformed to a nonlinear scale. This causes an asymmetrical frequency distribution of DT, erroneous estimation of the average growth rate, and sometimes contradictory results, compared to SGR. In addition, with limited number of tumor volume measurements, curve fitting of different growth models is not sufficient to estimate the true growth model. Analysis of the correlation between growth rate and the volume of tumor may give better estimate of tumor growth model for some types of tumors. Formation times and formation rates of metastases may also be estimated by the linear regression of SGR with the logarithm of tumor volume. Furthermore, tumor response was found to be equal to the logarithm of the ratio of post-treatment tumor volume to the volume of corresponding untreated tumor. Neglecting the natural growth characteristics of tumors results in underestimation of treatment effectiveness using the current routine criteria. The presented model may also facilitate integration of data from tumor size changes with data from functional imaging, e.g. PET or MRI, for therapeutic efficacy assessment.

In conclusion, SGR should replace DT for quantification of tumors growth rate. The relation between growth rate and tumor volume may facilitate estimation of non-exponential growth characteristics of tumors or metastatic dissemination rate. Tumor response to therapy can be assessed with a general continuous dimensionless quantity for both cytotoxic and cytostatic agents.

Keywords: tumor, growth, modeling, response, therapy

List of Papers

This work is based on four papers, which will be referred to in the text by their roman numbers.

Paper I

Mehrara E, Forssell-Aronsson E, Ahlman H, Bernhardt P. Specific growth rate versus doubling time for quantitative characterization of tumor growth rate. Cancer Research; 67(8): 3970-3975, 2007

Paper II

Mehrara E, Forssell-Aronsson E, Ahlman H, Bernhardt P. Quantitative analysis of tumor growth rate and changes in tumor marker level: Specific growth rate versus doubling time. Acta Oncologica; 48: 591-597, 2009

Paper III

Mehrara E, Forssell-Aronsson E, Johansson V, Kölby L, Ahlman H, Bernhardt P. Analysis of the growth model of solid tumors in clinical studies. Manuscript, 2010

Paper IV

Mehrara E, Forssell-Aronsson E, Bernhardt P. Objective assessment of solid tumor response to therapy based on tumor growth kinetics. Manuscript, 2010

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Preliminary results have been presented at:

Mehrara, E., E. Forssell-Aronsson and P. Bernhardt (2004). Modelling of metastatic tumour growth in the liver and its use for cancer treatment. Meeting of the Swedish Cancer Society, Gothenburg, Sweden

Mehrara, E., E. Forssell-Aronsson and P. Bernhardt (2005). Specific growth rate: A method to study tumour growth. European Conference on Mathematical and Theoretical Biology-ECMTB05. Dresden, Germany.

Mehrara, E., E. Forssell-Aronsson and P. Bernhardt (2006). Quantitative characterization of tumor growth rate. Meeting of the Swedish Cancer Society, Malmö, Sweden

Mehrara, E., E. Forssell-Aronsson and P. Bernhardt (2008). Specific growth rate (SGR) for quantitative analysis of tumor growth and response to therapy. Quantitative Imaging and Dosimetry symposium. Berder, France

Mehrara, E., E. Forssell-Aronsson, V. Johanson, L. Kolby, H. Ahlman and P. Bernhardt (2008). Estimation of tumour growth model, tumour formation time, and metastasis formation rate in clinical studies. ESTRO 27, Göteborg, Sweden.

Mehrara, E., E. Forssell-Aronsson and P. Bernhardt (2009). Assessment of solid tumor response to therapy in clinical trials. Meeting of the Swedish Cancer Society, Gothenburg, Sweden

Abbreviations

ADN	adenocarcinoma
BAC	bronchioalveolar carcinoma
CR	complete response
CLR	cell loss rate
CPR	cell proliferation rate
DT	doubling time
DT_e	equivalent doubling time
DT_{gm}	geometric mean doubling time
DT_{log}	antilog of mean of logarithms of doubling times
DT_m	arithmetic mean doubling time
DT_{true}	true doubling time
HCC	hepatocellular carcinoma
LR	log-ratio
MCP	metastatic cure probability
MLC	metastatic lung carcinoma
NSC	non-small cell carcinoma
NSCLC	non-small cell lung carcinoma
PC	pancreatic carcinoma
PD	progressive disease
PR	partial response
RECIST	response evaluation criteria in solid tumors
SCC	squamous cell carcinoma
SCLC	small cell lung carcinoma
SD	stable disease
SGR	specific growth rate
TR	tumor response

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Introduction

Modeling tumor growth

Modeling the natural (therapy-naive) growth of tumors is of value in the study of tumor progression, along with that it will be supportive for optimization of screening programs, prognostication (Bassukas, Hofmockel et al. 1996), optimal scheduling of chemotherapy (Norton 1988) and radiation therapy, and assessment of tumor spread (number and size distribution of metastases, including micro-metastases) (Iwata, Kawasaki et al. 2000; Withers and Lee 2006), information that is valuable for targeted radionuclide therapy (Williams, Duda et al. 1988; Withers, Peters et al. 1995; Bernhardt, Ahlman et al. 2003).

Tumor response to therapy may also be studied by analyzing the effect of therapy on the natural growth of tumor. However, there are mainly two types of growth models for tumors: exponential and non-exponential.

Exponential growth model

According to the Exponential growth model, tumor volume increases exponentially by time:

$$V=V_0e^{\alpha t}, \qquad (1)$$

where α is the exponential growth constant, and V and V_0 are the tumor volume at times t and 0, respectively. This model implies that the tumor volume can increase indefinitely and the growth rate of tumor is proportional to its volume:

$$\frac{dV}{dt} = \alpha V \qquad (2)$$

Tumor volume doubling time (DT)

Tumor volume doubling time, DT, was introduced for quantification of tumor growth rate in 1956 when Collins *et al.* proposed a graphical method to estimate the DT of tumors (Collins, Loeffler et al. 1956). DT is the time needed for a tumor to double in volume. The mathematical approach for estimation of DT was then proposed in 1961 (Schwartz 1961):

$$DT = \frac{(t_2 - t_1)\ln 2}{\ln(V_2/V_1)}, \qquad (3)$$

where V_1 and V_2 are two tumor volume estimates at two different occasions, t_1 and t_2, respectively. DT has been widely used as a quantity for tumor growth rate since its introduction. There are flaws with DT as a quantity for tumor growth rate: the frequency distribution of DT in a population is not normal and there are tumors with very long DT values in a population (Spratt 1969). Therefore, mean DT, DT_m, does not indicate the average growth rate and DT is not suitable for common statistical testing. Some researchers have approximated the frequency distribution of DT by a log-normal distribution (Spratt and Spratt 1964; Shackney, McCormack et al. 1978; Balmukhanov, Turdugulov et al. 1982; Kuroishi, Tominaga et al. 1990; Usuda, Saito et al. 1994). The average growth rate is then estimated by DT_{log}, calculated as the antilog to the arithmetic mean of the logarithms of doubling times (Spratt 1969; Gregory, Richards et al. 1991; Spratt, Meyer et al. 1995). The logarithm of DT, log(DT), is also proposed to be more suitable for statistical testing (Spratt 1969). DT_{log} is mathematically equal to geometric mean DT, DT_{gm}, which is also used to estimate the average growth rate (Kuroishi, Tominaga et al. 1990; Blomqvist, Wiklund et al. 1993; Usuda, Saito et al. 1994). It is also clear from Eq. 3 that DT is not defined when the estimated tumor volumes are equal. The reason for these flaws with DT has not previously been studied.

Specific growth rate (SGR)

From Eq. 2, the exponential growth constant, α, is equal to the specific growth rate, SGR, of tumor:

$$\mathrm{SGR} = \frac{1}{\mathrm{V}}\frac{\mathrm{dV}}{\mathrm{dt}} \qquad (4)$$

SGR is the relative change in tumor volume per unit time, and can be given as percent per unit time. For an exponentially growing tumor, SGR is a constant for each tumor, *i.e.*, SGR is independent of tumor volume or age. The exponential model can thus be rewritten as $SGR(t)=SGR_0$, where SGR_0 is the value of SGR at time $t_0=0$. More rapidly growing tumors have higher SGR values, SGR=0 represents non-growing tumors, and negative SGR values can be assigned to tumor regression.

According to Eq. 1 and Eq. 4, SGR of a tumor can be estimated with two volume measurements (V_1, V_2) at two different occasions (t_1, t_2):

$$SGR = \frac{\ln(V_2/V_1)}{t_2 - t_1} \quad (5)$$

From Eq. 3 and Eq. 5, the relation between SGR and DT is as follows:

$$DT = \frac{\ln 2}{SGR} \quad (6)$$

However, in the clinics and in clinical studies SGR is not known and tumor growth rate is usually quantified using DT. Accuracy of tumor growth rate quantities, SGR and DT, has not previously been studied.

Non-exponential tumor growth models

Studies have shown that tumor growth rate may decline with time (Hart, Shochat et al. 1998; Bajzer 1999; Afenya and Calderon 2000), which results in non-exponential growth model of tumors. Growth deceleration has been observed in animal models (Wennerberg, Willen et al. 1988), for solid tumors in clinical studies (Spratt, von Fournier et al. 1993; Spratt, Meyer et al. 1996), and in leukemia (Afenya and Calderon 2000). Growth deceleration is attributed to several factors, including prolonged cell cycle, reduced growth fraction, decreased availability of oxygen (Pavelic, Porter et al. 1978), decreased cell proliferation rate with increased cell loss rate (Bassukas and Maurer-Schultze 1987), tumor-related systemic factors (DeWys 1972), and allometric growth control (Prehn 1991). A number of non-exponential growth models are available in the literature, among which the Gompertzian model is widely used (Araujo and McElwain 2004).

The Gompertzian model

According to the Gompertzian growth model, the variation of tumor volume by time is as follows (Marusic, Bajzer et al. 1994; Afenya and Calderon 2000):

$$V = V_0 e^{\frac{\alpha}{\lambda}(1 - e^{-\lambda t})}, \quad (7)$$

where α is comparable with the growth constant in the exponential model, i.e. SGR at t=o, and λ is a constant for growth retardation. The Gompertzian model decreases to the exponential

model (Eq. 1) when λ approaches zero (λ→0). According to the Gompertzian model, the tumor cannot grow indefinitely, but asymptotically approaches a maximum equal to $V_0 e^{\frac{\alpha}{\lambda}}$, when t→∞.

Tumor growth model in clinical studies

The basic method to find the growth model of tumors is by direct curve fitting. Using this method, different growth model equations are fitted to the volume of each individual tumor and the model with the best fitting can be selected. In clinical studies, where the natural growth of tumor can be followed for a limited period, the exponential model is usually used to describe the growth of tumors. Proposals for new quantitative approaches to analyze tumor growth models in clinical settings are thus needed.

The SGR of different tumor types or even metastases of the same type, in the same patient, and in the same tissue are not necessarily the same. The variation of SGR among the tumors can be a result of biological differences between tumors, or growth retardation. If the growth model of tumors is the Gompertzian then larger tumors will have lower SGR values and vice versa. From Eq. 7, the relation between SGR and tumor volume is as follows:

$$SGR = SGR_0 - \lambda \ln\left(\frac{V}{V_0}\right) \qquad (8)$$

Eq. 8 shows that SGR decreases linearly by the logarithm of tumor volume if the growth model is Gompertzian. Eq. 8 does not include time, which makes it possible to use data from tumors without the knowledge of the age of each tumor. The feasibility of estimating the non-exponential growth parameter of tumors, i.e. λ in Eq. 7-8, based on growth rate relation with tumor volume needs to be investigated.

Tumor response to therapy

Assessment of tumor response to therapy is necessary for evaluation of the efficacy of novel anticancer drugs in clinical trials. It may also be valuable in individualized therapy rather than standardized treatment regimen in daily clinical practice. Traditional anticancer agents exhibit cytocidal effect by actively destroying tumor cells and, therefore, tumor shrinkage has been used as measure of treatment efficacy.

Response Evaluation Criteria in Solid Tumors (RECIST)

The Response Evaluation Criteria in Solid Tumors (RECIST) is currently adopted by academic and industrial groups (Miller, Hoogstraten et al. 1981; Therasse, Arbuck et al. 2000), where response to therapy is categorized as follows: Complete response (CR): the disappearance of all target lesions; Partial response (PR): at least a 30% decrease in the sum of the longest diameter of target lesions; Progressive disease (PD): at least a 20% increase in the sum of the longest diameter of target lesions or the appearance of one or more new lesions; Stable disease (SD): neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease (Therasse, Arbuck et al. 2000). Appropriateness of RECIST criteria, e.g. whether the change in tumor size is a proper endpoint for response assessment, has been widely discussed (Barnacle and McHugh 2006; Tuma 2006; Twombly 2006; Eisenhauer 2007).

Limitations of RECIST

The following four limitations can be identified in the RECIST criteria:

Firstly, the idea behind RECIST is that a treatment regimen is effective if the tumor size is reduced. However, there are emerging numbers of anticancer drugs, which inhibit tumor growth rather than actively destroy tumor cells. Traditional response evaluation criteria, including RECIST, may not be appropriate to assess the efficacy of such cytostatic agents, which do not result in tumor regression to a point of PR or CR. General means of assessment of both cytocidal and cystostatic effects must, therefore, be developed (Michaelis and Ratain 2006; Gwyther and Schwartz 2008).

Secondly, the natural growth rate of tumor is not considered in RECIST. A certain treatment that kills the same relative amount of tumor cells in two different tumor types will give different results, according to RECIST, if the natural proliferation rates of tumor cells are different.

Thirdly, adopting the RECIST criteria will convert a continuous variable, as tumor response, into a discrete variable; resulting in loss of information. This will make comparison of individual, or combination of, treatments less accurate. Furthermore, attempts to relate treatment efficacy to molecular or cellular characteristics of tumors, e.g. by systems biology approach, will be difficult when data is categorized.

Fourthly, many studies have shown that the effect of treatment on tumors can be assessed by means of changes in tumor characteristics other than size, e.g., estimated by positron emission

tomography (PET) or magnetic resonance imaging or spectroscopy (MRI/MRS). RECIST does not accommodate integration of physiological or functional imaging parameter into anatomical changes in tumor, and, therefore, new methods must be developed (Jaffe 2008).

The log-ratio (LR) method

Karrison et al. (Karrison, Maitland et al. 2007), based on suggestions by Lavin et al. (Lavin 1981), demonstrated that clinical trial designs that treat change in tumor size as a continuous variable rather than categorizing the changes are feasible (Karrison, Maitland et al. 2007). They used the logarithm of the ratio of tumor volume after therapy to that at baseline as a continuous end-point for quantification of tumor response, denoted as LR (log-ratio) (Karrison, Maitland et al. 2007).

Limitation of LR method

The main flaw with LR method is that the natural growth of tumor between measurement at baseline and therapy initiation and also during therapy is neglected.

Aims

The aims of this work were

1) To determine an accurate quantity for tumor growth rate. (Papers I, II)

2) To develop a method for estimation of tumor growth rate and dissemination rate in clinical settings. (Paper III)

3) To develop a general measure for assessment of tumor response to therapy, where the natural tumor growth rate is taken into account. (Paper IV)

Material and Methods

Quantitative analysis of tumor growth and dissemination

SGR versus DT: influence of measurement uncertainties (paper I)

To determine the most accurate quantity for tumor growth rate, equivalent doubling time, DT_e, was compared to DT. DT_e is DT calculated for mean SGR in a population. DT, log(DT), and SGR, the frequency distribution of these parameters and variation of their means, DT_m, DT_{log}, and DT_e, were analyzed by computer simulations and clinical data.

Monte Carlo simulations

Computer simulations were done using a Monte Carlo code, written in visual basic 6.0 (Microsoft, USA), for typical values of measurement time interval and DT (Table 1) (Rew and Wilson 2000).

For each time interval 10^5 simulations were done. In each simulation, V_1 and V_2 were generated and SGR_i and DT_i were estimated for the range of i indices 1-10^5. For each time interval, DT_m, DT_{log}, mean SGR ($\overline{SGR}$), and DT_e were calculated, where

$$\overline{SGR} = (SGR_1 + SGR_2 + \ldots + SGR_{100000})/100000 \qquad (9)$$

and

$$DT_e = \ln(2)/\overline{SGR}\,. \qquad (10)$$

The relative uncertainty of SGR $(\sigma_{SGR}/\overline{SGR})$ was calculated and compared to the expected uncertainty calculated from Eq. 4, which can be rewritten as $SGR = [\ln(V_2) - \ln(V_1)]/(t_2 - t_1)$, giving

$$\sigma_{SGR} = \sqrt{{\sigma_{\ln V1}}^2 + {\sigma_{\ln V2}}^2}\,/(t_2 - t_1) = \sqrt{(\sigma_{V1}/V_1)^2 + (\sigma_{V2}/V_2)^2}\,/(t_2 - t_1)\,. \qquad (11)$$

If both sides of the above equation are divided by SGR and SGR on the right side is replaced from Eq. 5, then:

$$\sigma_{SGR}/SGR = (1/\ln 2)\cdot\{DT/(t_2 - t_1)\}\cdot\sqrt{(\sigma_{V1}/V_1)^2 + (\sigma_{V2}/V_2)^2}\,. \qquad (12)$$

Since DT is inversely proportional to SGR, the simulation will generate unstable results for SGR close to zero. Therefore, SGR values between -0.0000693 and +0.0000693, corresponding to DT_i with absolute values longer than 10000 days, were excluded from the calculations.

Since the logarithm of negative and zero values are undefined, the following method was used in the calculation of DT_{log} in the presence of negative DT_i values: the absolute value of the minimum possible DT (-10000) plus one, *i.e.* 10001, was added to all DT values, and the mean of their logarithms was calculated. Thereafter, DT_{log} was derived by subtracting 10001 from the obtained mean value. To investigate how the exclusion of negative growth rate values can influence the average growth rate estimators, DT_m, DT_{log}, and DT_e, the simulation was then repeated excluding SGR values less than +0.0000693.

Clinical data

DT_m, DT_{log}, and DT_e were calculated for several types of tumors using quantitative data from previously published clinical studies (Table 1)(Blomqvist, Wiklund et al. 1993; Saito, Matsuzaki et al. 1998; Nishida, Kaneko et al. 1999; Wang, Sone et al. 2000; Furukawa, Iwata et al. 2001; Nakajima, Moriguchi et al. 2002; Winer-Muram, Jennings et al. 2002; El Sharouni, Kal et al. 2003). The results were then compared with computer simulations.

Table 1) Clinical data on tumor growth obtained from the literature. W, M, and P denote well, moderate, and poorly differentiated, respectively. n=number of tumors

Study	Tumor	Measurement time interval (d)	Doubling time range (d)	n	Reference
1	Pancreatic carcinoma	Not published	18-232	12	(Nishida, Kaneko et al. 1999)
2	Pancreatic carcinoma	99-751	64-255	9	(Furukawa, Iwata et al. 2001)
3	Adenocarcinoma (lung)	159-396	72-131	8	(Wang, Sone et al. 2000)
4	Adenocarcinoma (lung)	25-1212	(-1350)-964	15	(Winer-Muram, Jennings et al. 2002)
5	Bronchioalveolar (lung)	39-973	36-1092	9	(Winer-Muram, Jennings et al. 2002)
6	Squamous cell lung carcinoma	43-536	(-1214)-225	16	(Winer-Muram, Jennings et al. 2002)
7	Non small cell lung carcinoma	82-948	48-698	6	(Winer-Muram, Jennings et al. 2002)
8	Non small cell lung cancer	16-99	8-171	18	(El Sharouni, Kal et al. 2003)
9	Small cell lung cancer	299-386	54-132	4	(Wang, Sone et al. 2000)
10	Sarcoma (lung metastases)	14-819	7-1172	21	(Blomqvist, Wiklund et al. 1993)
11	Hepatocellular carcinoma (W)	43-252	38-274	19	(Nakajima, Moriguchi et al. 2002)
12	Hepatocellular carcinoma (W)	63-763	76-720	15	(Saito, Matsuzaki et al. 1998)
13	Hepatocellular carcinoma (M)	13-224	17-91	9	(Nakajima, Moriguchi et al. 2002)
14	Hepatocellular carcinoma (M)	91-210	94-380	6	(Saito, Matsuzaki et al. 1998)
15	Hepatocellular carcinoma (P)	20-182	20-78	6	(Nakajima, Moriguchi et al. 2002)

SGR versus DT regardless of measurement uncertainties (paper II)

Variation of DT per unit SGR

According to Eq. 6, the variation of DT with SGR is:

$$\frac{\Delta \mathrm{DT}}{\Delta \mathrm{SGR}} = \frac{\ln(2)}{\mathrm{SGR}^2} \qquad (13)$$

It shows that the variation of DT per unit SGR is not constant for the whole range of SGR; it quickly decreases with increasing the absolute value of SGR. Variation of DT per unit SGR was plotted for SGR values between -5 %/d and +5 %/d, corresponding to DT values of -14 days to -∞ and 14 days to +∞, respectively.

Clinical data

Two examples from previously published articles were found that could represent the difference between the results of statistical analyses based on DT and SGR. In the first study, the authors found statistically significant difference between DT of prostate specific antigen (PSA) before and after treatment in each of 9 out of 12 patients (Guess, Jennrich et al. 2003). Using the signed rank test, they could also detect a significant positive shift in the frequency distribution of DT after treatment. In the present study, increase rate of PSA before and after treatment was compared in 12 patients using DT as well as SGR of PSA by student's t-test (Table 2). (Note: The authors of the original article used a method to study PSA level variations in each patient, while in the current study the PSA change in the group of patients was studied). In the second example, the authors examined the DT of serum CA 19-9 in patients with pancreatic cancer (Nishida, Kaneko et al. 1999). A significant correlation was found between the DT of the serum level of CA 19-9 and the DT of tumor volume in 11 out of 75 patients, where both DT values were available. In the present study, the corresponding SGR values of the DT of tumor marker as well as the DT of tumor volume were calculated (Table 3) and the correlation between the two variables was examined.

Analysis of tumor growth in clinical settings (paper III)

Clinical data

Data from population studies

Data from clinical studies were retrieved from the literature based on the availability of tumor volume estimates and corresponding measurement time intervals. Correlation between the growth rate and the volume of tumor was calculated for the following types of tumors: meningioma (Nakamura, Roser et al. 2003; Nakasu, Fukami et al. 2005), hepatocellular carcinoma (Saito, Matsuzaki et al. 1998; Nakajima, Moriguchi et al. 2002; Taouli, Goh et al. 2005), pancreatic carcinoma (Furukawa, Iwata et al. 2001), and primary lung cancer (Wang, Sone et al. 2000).

Data from individual patients

The first patient was diagnosed with primary midgut carcinoid and liver metastases. The primary tumor was surgically resected in 1995. Growth data were obtained from 8 CT examinations performed annually during 1995-2002. During this period the patient was treated with octreotide (Sandostatin, Sandoz/Novartis, Basel, Switzerland) for hormonal symptom

relief, and interferon alfa-2b (IntronA, Schering-Plough Corporation, New Jersey, USA) was given at three occasions without clinical response. The volume of each tumor was measured by point counting: a transparent paper with square millimeters was used to measure the tumor area in CT slices and the tumor volume in the slice was estimated by multiplying the tumor area and the slice thickness. The total volume of the tumor was calculated as the sum of tumor volumes from the different CT slices.

The second patient was diagnosed with primary renal cell carcinoma with lung metastases. The growth of 7 lung metastases in this untreated patient was studied. A total of 32 conventional two-dimensional AP chest radiographs from the patient were available from 1989 to 1999. The area of each tumor in each radiograph was then estimated using Osiris (cf. http://www.sim.hcuge.ch/osiris/01_Osiris_Presentation_EN.htm). Each tumor was assumed to be equal to the volume of a sphere with the diameter of a circle with the same area as the estimated tumor area in the radiograph. Since the lining border of the tumor could not be clearly defined in all images, the number of available data points may be different for different metastatic tumor masses.

Direct curve fitting

The exponential (Eq. 1) and the Gompertzian (Eq. 7) growth curves were fitted to the volume of any metastases in individual patients. The curve fittings were performed using Matlab 6.5.1 with the curve fitting toolbox (The MathWorks, USA).

SGR deceleration analysis

Correlation between tumor growth rate and its volume for each group of tumors was studied as follows. (1) SGR values were calculated according to Eq. 5 for each pair of consecutive tumor volume measurements. (2) Each SGR value was assigned to the geometric mean of the two volumes. (3) Correlation between SGR and the logarithm of tumor volume was calculated for all SGR values for the same tumor type in a population or for the same type of metastasis in the same host tissue in the same patient.

The relatively large number of tumors in one of the meningioma studies (Nakamura, Roser et al. 2003) enabled us to draw the frequency distribution of tumors in small (< 6 cm^3) and large (>6 cm^3) groups and compare SGR between the groups using Student's t-test.

Metastasis formation rate estimations

For data from individual patients (two cases), the analysis was continued as follows. (1) Assuming $V_0=10^{-9}$ cm^3 (one cell), Eq. 7 with parameters obtained from the regression line between SGR and the logarithm of tumor volume was assumed to represent the general Gompertzian growth model of the metastases (with variable formation time). Formation time of each tumor was obtained by the best fitting of the general growth model to the volume of the tumor. (2) Formation time of the earliest metastasis was set to zero and the number of metastases as a function of time after formation of the first metastasis was obtained. (3) To study the applicability of the standard curve fitting method, the exponential and the Gompertzian curves were fitted to the growth of each tumor and the best fits were estimated. The metastasis formation rate was calculated as above according to the best exponential fit to each tumor.

Modeling tumor response to therapy (paper IV)

Kinetics of tumor growth

Eq. 5 can be used to estimate the SGR of tumors at any time period. If SGR is time dependent, as for non-exponentially growing tumors, Eq. 5 can be rewritten as follows:

$$\ln\left(\frac{V}{V_0}\right)=\int_{t_0}^{t} SGR(t)dt\,, \qquad (14)$$

where SGR(t) is the SGR at time t. The value of SGR(t) depends on the level of cell proliferation rate, CPR(t), and cell loss rate, CLR(t), at time t:

$$SGR(t)=CPR(t)-CLR(t) \qquad (15)$$

Tumor response to therapy (TR)

If the natural growth of tumor is interrupted by therapy, an effective therapeutic agent may increase the CLR (cytotoxic effect) and/or decrease the CPR (cytostatic effect) of tumor. An effective treatment will thus decrease SGR to SGR′ regardless of the mechanism of the therapeutic effect:

$$SGR'(t)=SGR(t)-\Delta SGR(t)\,, \qquad (16)$$

where ΔSGR(t) is the effect of treatment at time t. Temporal variation of SGR′ depends on all factors that naturally affect tumor growth as well as the effect of therapy. Readjustment and integration of the above equation over time gives:

$$\int_{t_i}^{t} \Delta SGR(t) \cdot dt = \int_{t_i}^{t} SGR(t) \cdot dt - \int_{t_i}^{t} SGR'(t) \cdot dt \ ,$$

where t_i and t are the time of therapy initiation and efficacy assessment, respectively. The right side of the above equation can be replaced using Eq. 14, which gives:

$$\int_{t_i}^{t} \Delta SGR(t) \cdot dt = \ln(\frac{V_n}{V_i}) - \ln(\frac{V_t}{V_i}) \ ,$$

where V_i is tumor volume at time of therapy initiation, and V_t and V_n are the volume of treated and corresponding (hypothetical) non-treated tumor at time of efficacy assessment, respectively. The left side of the above equation is the overall effect of treatment during time from treatment initiation to time of efficacy assessment, and can be denoted as TR (tumor response). Since $\ln(V_n/V_i)-\ln(V_t/V_i)=-\ln(V_t/V_n)$:

$$TR=-\ln\left(\frac{V_t}{V_n}\right) \qquad (17)$$

Based on the above equation, TR is a general continuous dimensionless quantity for tumor response to both cytotoxic and cytostatic therapeutic effects. TR can thus be calculated by the logarithm of the ratio of post-treatment volume of tumor to the volume that the tumor would have (at time of efficacy assessment) if the growth was not interrupted by therapy. The value of V_n can be estimated having the natural growth model of tumor.

Eq. 17 was transformed by replacing V_n with the following assumptions: (1) Tumor volume at first diagnostic investigation is V_d; (2) therapy is initiated Δt_{pre} days after measurement of V_d; (3) tumor grows exponentially with $SGR(t)=SGR_0$ during this period and tumor volume at time of therapy initiation is V_i; (4) tumor response is assessed Δt_{post} days after therapy initiation and

tumor volume at time of efficacy assessment is V_t; (5) tumor would continue to grow with SGR_0 if the growth was not interrupted and its volume would be V_n at time of efficacy assessment.

Non-Hodgkin's lymphoma patients treated with ^{131}I labeled anti-B1 antibody

TR values were calculated for treatment of non-Hodgkin's lymphoma patients with ^{131}I labeled anti-B1 antibody, where data was from a previously published article (Sgouros, Squeri et al. 2003). The study was selected based on the availability of tumor volumes and the time of pre- and post- treatment volume estimations in each patient: information which is necessary for TR calculation. Total tumor burden was assessed by drawing contours around all lymphoma lesions identified on whole-body CT or MRI. Variations of total tumor burden in 11 patients before and after treatment were estimated from figure 2 in the original article (Sgouros, Squeri et al. 2003). Two more patients are included in the original article, where tumors disappeared after treatment. Those data were excluded in the present study. To estimate the natural growth rate of Non-Hodgkin's lymphomas in the present study the average post-treatment re-growth rate of 5 tumors was used.

Results

Tumor growth and dissemination

SGR versus DT: influence of measurement uncertainties (paper I)

Monte Carlo simulations

Figure 1 shows the simulated frequency distributions of DT (panel A), log(DT) (panel B), and SGR (panel C) for different time intervals (1, 5, 10, 50, 100, and 200 days), when the relative uncertainty of the volume measurement was 10%. For a time interval of 200 days (2 DT_{true}), all DT values were positive and the frequency distribution of DT was symmetric and centered at DT=100 days (Fig. 1A). When the time interval was 100 days (1 DT_{true}) the frequency distribution of DT was positively skewed and the peak shifted towards lower DT values. When the time interval was 50 days (0.5 DT_{true}) the peak shifted more towards lower DT values and negative DT values appeared in the data as a very small peak in the negative range. The peak in the negative range increased further with decreasing time interval. With a 1 day time interval the two peaks were very close and symmetric in relation to zero and appeared as a single peak centered at zero (Fig. 1A). Therefore, mean DT was close to zero for very short time intervals. Theoretically, when the time interval approaches zero the position of two peaks asymptotically approaches zero with a height of infinity. If negative values of DT were excluded, the peaks on the negative side of the frequency distribution of DT disappeared. Variations in the frequency distribution of log(DT) (Fig. 1B) were comparable to that of DT. For the time intervals of 200 and 100 days all DT values were positive and only one peak appeared in the frequency distribution of log(DT) centered at 4.6 (=log 100) for 200 days and slightly shifted to the left for 100 days. For shorter time intervals, where negative DT values appeared in data, the peak shifted more to lower values in relation to 4.6, when negative DT values were excluded (Fig. 1B). When negative DT values were included for 50, 10, and 1 day time intervals the symmetry point was shifted to 9.21 (=log 10001), *see* Materials & Methods, comparable to zero in the frequency distribution of DT (Fig. 1B-insert b). For a 1 day time interval the two peaks looked like a single peak centered at 9.21. Therefore, also DT_{log} was close to zero for very short time intervals (Fig. 2). The frequency distribution of SGR was symmetric for all time intervals studied. The mean SGR was equal to the true SGR (0.7 %/d) and its uncertainty increased with decreased time interval. The expected uncertainty of SGR from Eq. 12 and the calculated uncertainty of SGR from the simulations were well correlated (R^2>0.999).

The results of the computer simulations of DT_m, DT_{log}, and DT_e are shown in Figure 2. When the time interval was very long compared to DT_{true}, all DT estimators were equal to DT_{true} of the tumor (Fig. 2A). When the time interval decreased, DT_m overestimated DT_{true} with a maximum deviation of about 30%. For very short time intervals compared to DT_{true}, DT_m underestimated DT_{true} and approached zero for time intervals down to a few days. DT_{log} showed a similar

variation as DT_m, with a maximum overestimation of about 20%, but a larger underestimation than DT_m for short time intervals. Neglecting small fluctuations at very short time intervals, DT_e was equal to DT_{true} for all time intervals studied (Fig. 2A). When the negative growth rate values were excluded, DT_m and DT_{log} followed a similar shape as when the negative values were included (Fig. 2B), *i.e.*, overestimation up to a maximum and then decreasing with decreasing time interval. However, the ranges of deviation from DT_{true} were different. When negative values were excluded DT_m was much higher, while DT_{log} was closer to when negative values were included. Furthermore, DT_e decreased with decreasing time interval and was lower than DT_{log}, which in turn was lower than DT_{true} when negative values were excluded. Then, DT_e approached zero for very short time intervals down to a few days.

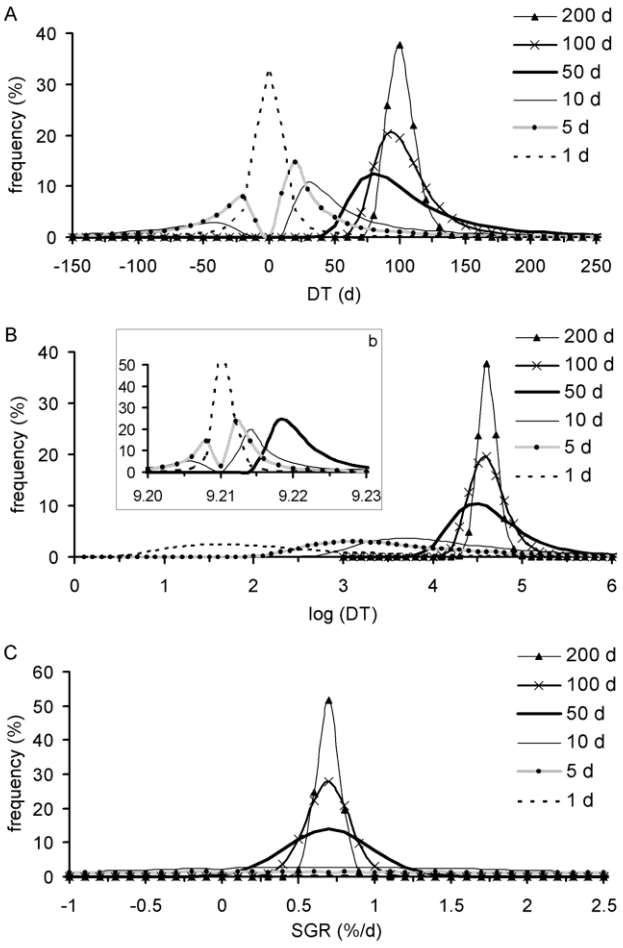

Figure 1) Simulated frequency distributions of A) DT, B) log(DT), and C) SGR at measurement time intervals from 1 to 200 days. The relative uncertainty of volume estimation was 10% and the true DT was 100 days. For 50, 10, and 1 day measurement time intervals, negative DT values were excluded for panel B, while included for the insert b and panels A and C. Note the different scales of the y-axes of all panels, and the different scales of the x-axes of Figure 2B.

Clinical data

DT_m, DT_{log}, and DT_e values estimated from the previously published clinical data on several types of tumors are presented in Figure 3. The measurement time intervals varied between 13 and 1212 days. The estimated doubling times from these papers were between -1350 and 1172 days. The only study containing negative growth rates was that of adenocarcinoma and squamous cell lung carcinoma (31). For all studies including only positive growth rates, DT_e was lower than DT_{log}, which was lower than DT_m (Fig. 3A). On average, DT_{log} and DT_m were 25% (range 3-88%) and 76% (range 6-317%) higher than DT_e, respectively. If the negative growth rates were included, negative DT_m was obtained, while DT_e was still positive (data not shown).

The SGR values from clinical data are summarized in Fig. 3B. Since SGR and DT are reciprocally related (Eq. 6), a higher SGR value in Fig. 3B corresponds to a shorter DT_e in Fig. 3A and vice versa. Such trend was not always seen for DT_m and DT_{log} values, since they may over- or underestimate the true DT of tumors depending on volume measurement uncertainties and the time interval.

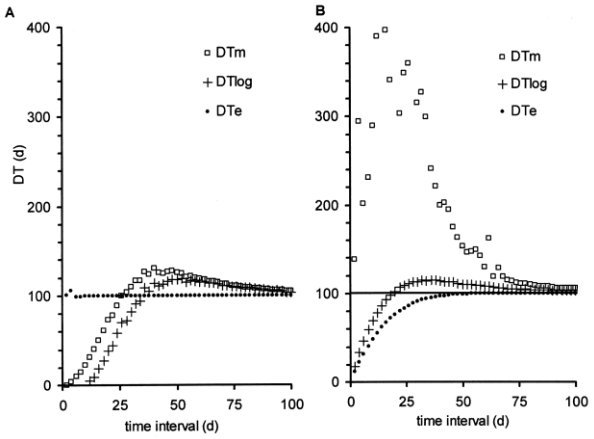

Figure 2) Simulated results of DT_m, DT_{log}, and DT_e for maximum 10% volume measurement uncertainty and different measurement time intervals. The true DT value was 100 days. Negative values of growth rate were included in panel A and excluded in panel B. For proper scaling of the DT axis and clear presentation of deviations from true DT, DT_m values of 454, 471, and 776 at 28, 20, and 14 days time intervals were excluded from panel B, respectively.

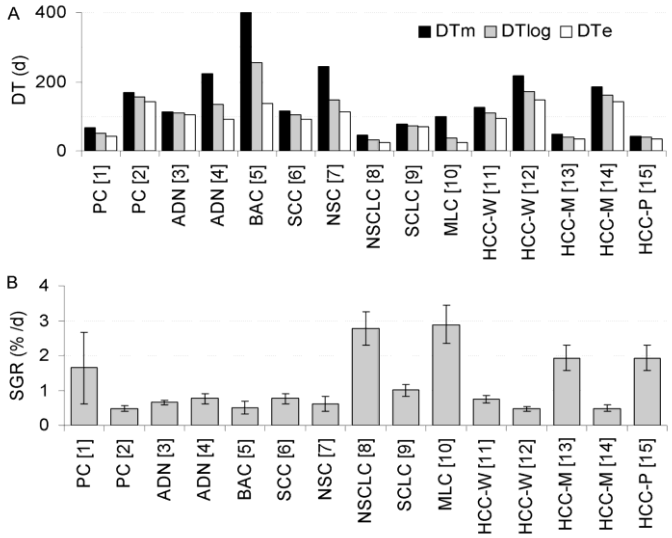

Figure 3) A) DT_m, DT_{log}, and DT_e and B) SGR values determined from previously published clinical data. Numbers in brackets show the study number according to Table 1. Abbreviations: PC: pancreatic carcinoma (Nishida, Kaneko et al. 1999; Furukawa, Iwata et al. 2001). Primary lung cancers: ADN: adenocarcinoma (Wang, Sone et al. 2000; Winer-Muram, Jennings et al. 2002), BAC: bronchioalveolar (Winer-Muram, Jennings et al. 2002), SCC: squamous cell carcinoma (Winer-Muram, Jennings et al. 2002), NSC: non-small cell carcinoma (Winer-Muram, Jennings et al. 2002), NSCLC: non-small cell lung cancer (El Sharouni, Kal et al. 2003), SCLC: small cell lung cancer (Wang, Sone et al. 2000). MLC: metastatic lung cancer from bone and soft tissue (Blomqvist, Wiklund et al. 1993). HCC: hepatocellular carcinoma (Saito, Matsuzaki et al. 1998; Nakajima, Moriguchi et al. 2002). W, M, and P denote well, moderate, and poorly differentiated tumors, respectively.

SGR versus DT regardless of measurement uncertainties (paper II)

Variation of DT per unit SGR

Figure 4 shows the variation of DT per 1 %/d change in SGR based on Eq. C. Each %/d of SGR corresponds to a change in DT of 3 days when the SGR is ±5 %/d. With decreasing the absolute value of SGR, each %/d change of SGR corresponds to a higher value on the DT scale, with 69 days at ±1 %/d and approaching infinity at SGR=0. A DT of 1 day does not represent the same growth rate when the tumor is slowly growing as when the tumor is rapidly growing (Fig. 4). For a slowly growing tumor with low SGR, DT increases considerably with a slight decrease in SGR. For a rapidly growing tumor with high SGR, DT decreases slightly even with a large increase in SGR. DT understates the growth rate of slowly growing tumors and overstates the growth rate of rapidly growing tumors.

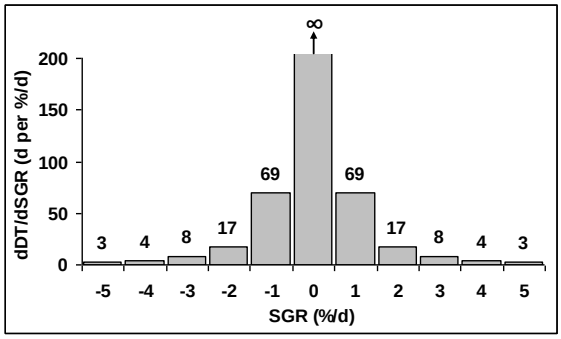

Figure 4) Change in DT per %/d change in SGR, dDT/dSGR, versus SGR. dDT/dSGR changes slightly for rapidly growing tumors, whereas it changes largely for slowly growing tumors and approaches ∞ when SGR approaches zero, *i.e.*, DT approaches ∞.

Table 2) Increase rate of PSA level before and after treatment initiation. DT values were retrieved from a previously published clinical study, Guess et al. (2003) (Guess, Jennrich et al. 2003), and the corresponding SGR values were calculated (Eq. 6). The difference between DT of PSA level before and after treatment is not statistically significant ($p>0.1$). The difference between SGR of PSA level before and after treatment is statistically significant ($p<0.002$). Note: Patient ID is the same ID used in the original paper.

PatientID	DT (months)		SGR (%/month)	
	Before treatment	After treatment	Before treatment	After treatment
3	3.97	13.43	17.46	5.16
7	5.67	10.11	12.22	6.86
8	1.14	2.91	60.80	23.82
9	3.37	7.71	20.57	8.99
11	1.58	16.49	43.87	4.20
13	10.5	7.97	6.60	8.70
15	2.66	11.95	26.06	5.80
17	3.64	3.27	19.04	21.20
18	2.04	4.96	33.98	13.97
20	2.33	3.24	29.75	21.39
21	6.29	-155.49	11.02	-0.45
22	5.12	-645.51	13.54	-0.11

Clinical data

For the clinical studies, the difference between DT of PSA level before and after treatment was not statistically significant ($p>0.1$), but the difference between SGR of PSA level before and after treatment was statistically significant ($p<0.002$) (Table 2). In addition, the correlation

between DT of CA 19-9 level and DT of tumor volume was statistically significant ($p<0.0001$), but the correlation between SGR of CA 19-9 and SGR of tumor volume was not statistically significant ($p>0.3$) (Table 3).

Table 3) Growth rate of tumor volume as well as the increase rate of serum CA 19-9 in 11 patients with pancreatic cancer. DT values were retrieved from a previously published clinical study, Nishida et al. (1999) (Nishida, Kaneko et al. 1999), and the corresponding SGR values were calculated (Eq. 6). The correlation between DT of CA 19-9 level and DT of tumor volume is statistically significant ($p<0.0001$). The correlation between SGR of CA 19-9 and SGR of tumor volume is not statistically significant ($p>0.3$). Note: Patient no. is the case number used in the original paper.

Patient no.	CA 19-9 DT (d)	Tumor DT (d)	CA 19-9 SGR (%/d)	Tumor SGR (%/d)
2	8.3	34.8	8.4	2.0
6	39.7	44.6	1.7	1.6
9	46.3	34.5	1.5	2.0
26	36.5	21.2	1.9	3.3
35	30.4	47.7	2.3	1.5
36	67.1	112.8	1.0	0.6
40	44.7	70.6	1.6	1.0
47	24.7	18.4	2.8	3.8
50	42.7	50.6	1.6	1.4
62	137.5	231.6	0.5	0.3
68	42.3	39.3	1.6	1.8

Analysis of tumor growth in clinical settings (paper III)

Tumors growth deceleration in the population data

The correlation between SGR and the logarithm of the volume was statistically significant for one study on meningiomas (Nakamura, Roser et al. 2003) and was not statistically significant for any of the other studies (Table 4). The difference between the growth rates of the large and small meningioma tumors with significant SGR deceleration was statistically significant ($p<0.001$), with higher SGR for smaller tumors (Fig. 6).

Table 4) Correlation between the specific growth rate, SGR, and the logarithm of tumor volume in groups of patients diagnosed with the same type of tumor. n: number of tumors. r: correlation coefficient. NS: not statistically significant

Tumor type (reference)	n	r^2	r	p-value
Meningiomas (Nakamura, Roser et al. 2003)	41	0.2424	-0.4923	<0.01
Meningiomas (Nakasu, Fukami et al. 2005)	36	0.0104	-0.1020	NS
Hepatocellular carcinoma (Nakajima, Moriguchi et al. 2002)	34	0.0380	0.1949	NS
Hepatocellular carcinoma (Saito, Matsuzaki et al. 1998)	21	0.0134	-0.1158	NS
Hepatocellular carcinoma (Taouli, Goh et al. 2005)	16	0.0014	-0.0374	NS
Pancreatic carcinoma (Furukawa, Iwata et al. 2001)	9	0.0041	-0.0640	NS
Primary lung cancer (Wang, Sone et al. 2000)	12	0.1619	-0.4024	NS

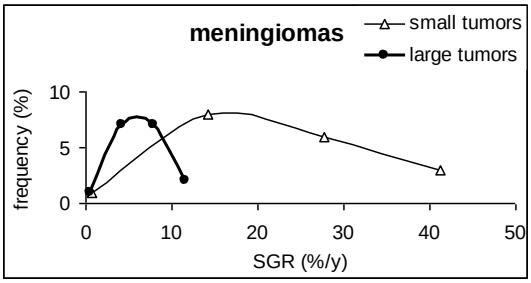

Figure 6) Frequency distribution of SGR in two groups of small (n=20) and large (n=21) meningioma tumors, based on data from reference (Nakamura, Roser et al. 2003). Mean SGR was 20 %/y and 6 %/y for small and large tumors, respectively.

Tumors growth models in individual patients

For the patient with liver metastases from a primary midgut carcinoid and the patient with lung metastases from a primary renal cell carcinoma, it was possible to examine direct curve fitting for most tumors, because the tumors had been followed for relatively long periods: up to 7 and 10 years, respectively. The volume of each tumor in the liver (except for two metastases), or in the lungs, could be well described either by the exponential or by the Gompertzian model. The two metastases in the liver were only observed at two occasions, and the Gompertzian model requires three data points for curve fitting. Based on the results of the direct model fitting it was not possible to select the most probable growth model of each tumor. However, the estimated tumor formation times and SGR_0 values were different when estimated by the different models. The estimated formation time of one of the tumors in the liver, obtained by the exponential fit, was not realistic (5 years before the birth of the patient). For the best

exponential fits the SGR values were 0.14-0.33 %/d. These values correspond to DT values of 7-17 months.

Figure 7 shows the best exponential and Gompertzian model curve fits for the volume of the largest metastasis in the liver. It shows that the two models could fit well with the volume of tumor in a short time interval, whereas the extrapolated formation times of tumor differ widely: 1947 and 1982 with the exponential and Gompertzian models, respectively. The estimated SGR at time of formation of tumor (SGR_0) was also largely different: 0.14 %/d and 1.1 %/d with the exponential and the Gompertzian models, respectively. These values correspond to DT values of 17 months and 2 months, respectively.

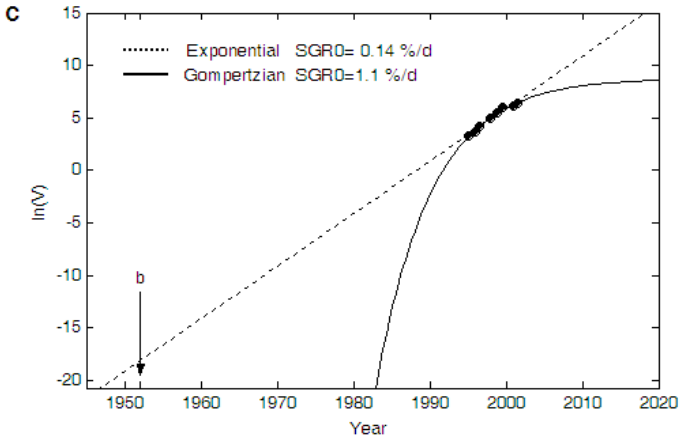

Figure 7) The best exponential (dashed line) and Gompertzian (solid line) model curve fits to the logarithm of the volume of metastasis A in the liver with extrapolation to the volume of one cell. b is the birth of patient.

The negative correlation between SGR and the logarithm of tumor volume was statistically significant (r^2=0.33, p<0.005) for the liver metastases, and the estimated λ and SGR_0 values were 0.00023 and 0.79 %/d, respectively. Curve fitting of the general Gompertzian growth model to data for the liver metastases are shown in Fig. 8. The same growth curve is shifted in time to fit the volume of each tumor.

For the patient with lung metastases, the SGR values for the best exponential fits were 0.14-0.39 %/d. These values correspond to DT values of 6-17 months, respectively.

For this patient the negative correlation between SGR and the logarithm of tumor was not statistically significant. The estimated λ and SGR_0 values were 0.00007 and 0.46 %/d, respectively. However, the general Gompertzian growth model based on these parameters

could be fitted to data from each metastasis in the patient. The same growth curve could be shifted in time to fit the volume of each tumor, as for liver metastases (Fig. 8).

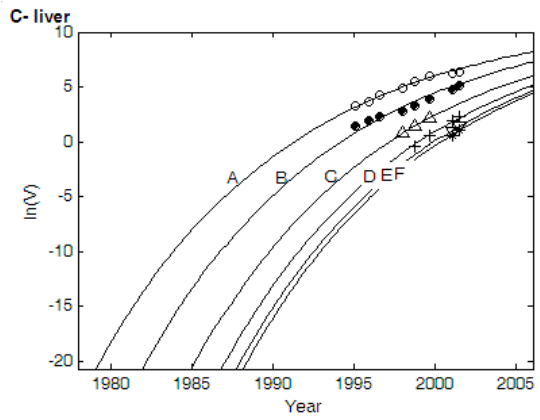

Figure 8) The logarithm of the tumor volume vs. time for all metastases in the liver with the general Gompertzian growth model curve fits.

Metastasis formation rates in individual patients

Figure 9 shows the number of metastases as a function of time in each patient. The number of metastases increased exponentially by time assuming that the tumors grow either exponentially with different growth rates or according to a general Gompertzian model. The increase rate of the number of metastases based on the Gompertzian model was higher than the rate based on the exponential model.

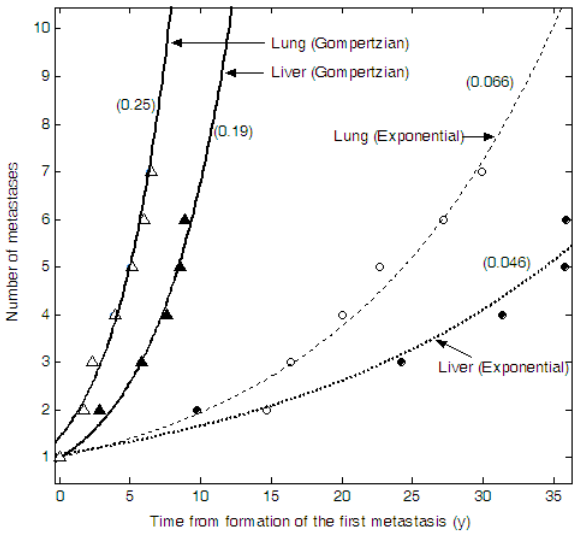

Figure 9) The number of metastases vs. the time from formation of the first metastasis. The metastasis formation rates were determined for the metastases in the liver and the lungs according to the exponential and Gompertzian growth models. Values in parentheses show the constant of exponential increase rate (y^{-1}).

Tumor response model (paper IV)

General tumor response model for exponentially growing tumors

When the tumor response model developed in the present study (Eq. 17) is applied to an exponentially growing tumor, TR is related to tumor volume and growth rate as follows (Fig. 10):

$$TR = \underbrace{-\ln\left(\frac{V_t}{V_d}\right)}_{LR} + \underbrace{\underbrace{SGR_0 \cdot \Delta t_{pre}}_{e1} + \underbrace{SGR_0 \cdot \Delta t_{post}}_{e2}}_{Err} . \quad (18)$$

The first term on the right hand side of the above equation, LR, is the treatment effectiveness where the natural growth of tumor is neglected and is equivalent to the log ratio (LR) measure suggested by Kharison et al. (Karrison, Maitland et al. 2007). LR values less than -0.5, between -0.5 and +1, and larger than +1 correspond to progressive disease, stable disease, and partial response according to RECIST, respectively. The second term, e1, and the third term, e2, represent tumor growth prior to and after treatment initiation, respectively. The overall effect of tumor growth from time of diagnosis to time of efficacy assessment, Δt, sums up as follows:

$$TR = -\ln\left(\frac{V_t}{V_d}\right) + SGR_0 \cdot \Delta t = LR + Err \quad (19)$$

The above equation indicates that evaluation of treatment effectiveness by comparing the volume of treated tumor with pre-treatment tumor volume underestimates the effect of therapy by Err.

Tumor response to pure cytostatic effect

If a therapeutic drug has pure cytostatic effect, i.e., the drug inhibits tumor growth, but does not destroy existing tumor cells, and if the drug can completely block tumor growth, the tumor volume at time of efficacy assessment will be the same as the tumor volume at time of treatment initiation, V_i. The cytostatic efficacy of treatment is then $e2=\ln(V_n/V_i)$ (Fig. 10). If the drug can partially control tumor growth, the tumor volume at time of efficacy assessment will be larger than V_i (closer to V_n) and the treatment efficacy will be less than e2 in Fig. 10. Note that tumor volume at time of efficacy assessment is, however, larger than tumor volume at time of diagnosis, V_d. According to RECIST, a V_t of more than $1.73V_d$ (20% increase in diameter) will be considered as progressive disease. For a tumor with doubling time shorter than 27 days

(SGR>2.6 %/d) and a treatment that completely blocks tumor growth, the drug will be considered without any effect and be categorized as progressive disease according to RECIST.

Non-Hodgkin's lymphoma patients response to therapy

Estimated TR values of the applied treatment for the patients are shown in Table 5. Assuming a treatment that has only pure cytostatic effect, i.e. $V_t=V_i$, TR values were also calculated. Based on mean and standard deviation values from data in Table 5, frequency distribution of TR and LR were approximated with corresponding normal distribution with the same mean and standard deviation values. These distributions are shown in Fig. 11A-B for the observed TR and LR values as well as TR and LR values calculated for pure cytostatic treatment, respectively.

Table 5) Treatment efficacy values for non-Hodgkin's lymphoma patients treated with ^{131}I labeled anti-B1 antibody, retrieved from ref. (Sgouros, Squeri et al. 2003) and treatment efficacy calculated for a pure cytostatic treatment that can completely block tumor growth.

Patient no.	Treatment efficacy (observed)			Treatment efficacy (calculated for a hypothetical pure cytostatic effect)		
	TR	LR	Category according to RECIST	TR	LR	Category according to RECIST
1	2.65	0.73	SD	1.26	-0.31	SD
2	7.42	2.56	PR	2.68	-0.79	PD
3	5.15	1.00	SD	1.57	-0.79	PD
4	4.451	2.4	PR	0.94	-0.24	SD
5	2.80	1.39	PR	0.94	-0.16	SD
6	4.50	-0.33	SD	2.83	-1.26	PD
7	6.38	2.13	PR	1.42	-0.63	PD
8	2.60	0.37	SD	1.26	-0.47	SD
9	3.18	1.17	PR	1.10	-0.31	SD
10	2.14	-0.46	SD	1.42	-0.79	PD
11	4.52	0.32	SD	0	-0.94	PD

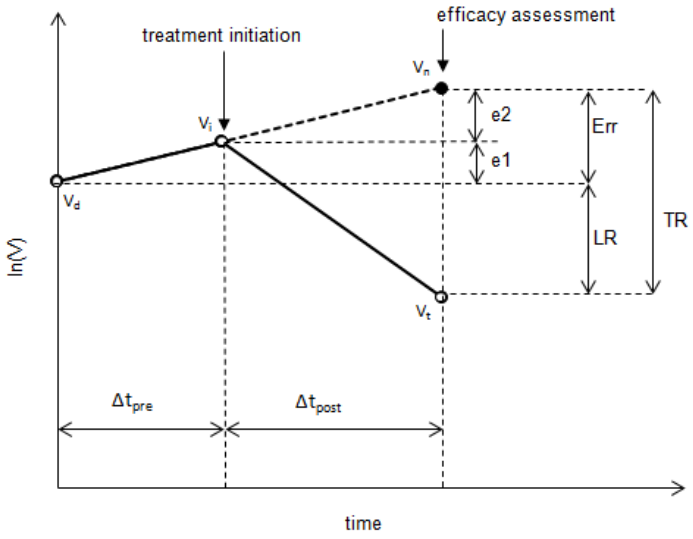

Figure 10) Variation of the volume of a hypothetical exponentially growing tumor before and after treatment. TR=tumor response, LR=log-ratio measure for treatment effectiveness based on ref. (Karrison, Maitland et al. 2007), e1 and e2= underestimation of TR if pre-treatment or post-treatment growth of tumor is neglected, respectively. Err=overall underestimation of TR if pre-treatment and post-treatment growth of tumor are neglected (TR=LR+e1+e2).

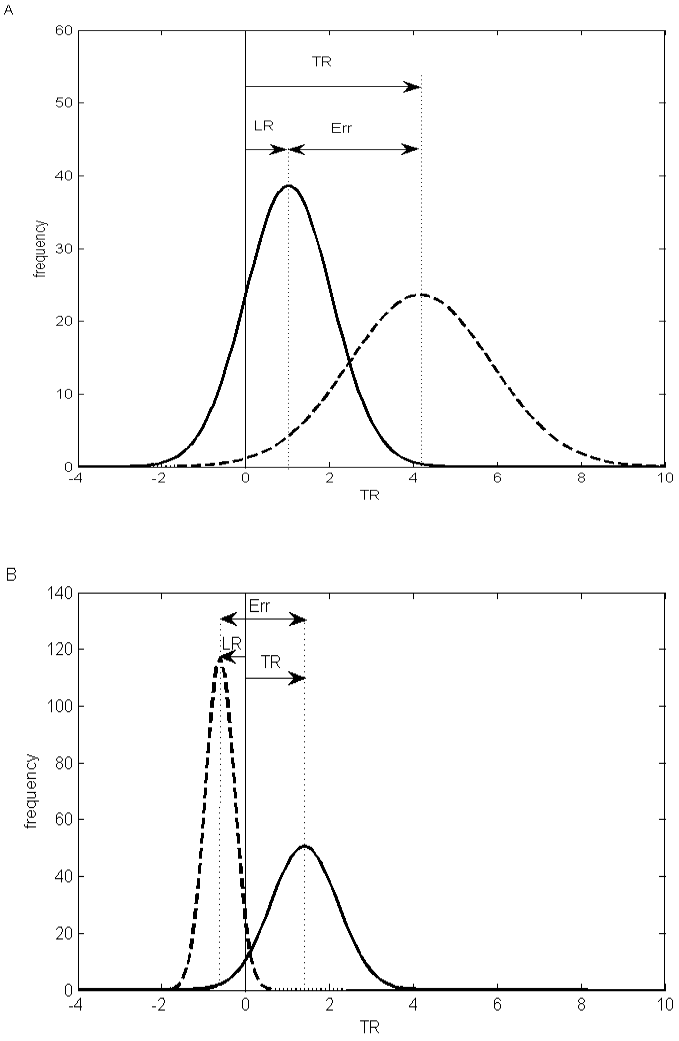

Figure 11) A) Frequency distribution of TR and LR values based on the treatment results of 11 non-Hodgkin's lymphoma patients. The mean and standard deviation values were obtained from the sample and the corresponding normal distributions were drawn. TR: mean=4.16, standard deviation=1.69, LR: mean= 1.02, standard deviation=1.03. B) Frequency distribution of treatment efficacy for the same data as panel A by assuming that a treatment has only cytostatic effect. TR: mean=1.4, standard deviation=0.69. LR: mean= - 0.61, standard deviation=0.34.

Discussion

SGR versus DT (Paper I, II)

The results clearly show that selecting a proper variable for tumor growth rate is crucial. Recalculation of the previously published clinical data, as well as computer simulations and mathematical analysis, showed that quantitative analyses based on tumor growth rate can lead to erroneous and even contradictory results depending on the variable used for growth rate: SGR or DT.

The quantity of tumor growth rate is used in a wide range of studies, e.g., when classifying tumors according to their growth rate (Arai, Kuroishi et al. 1994), or when the correlation between tumor growth rate with other factors is studied, e.g. patient survival (Balmukhanov, Turdugulov et al. 1982; Kuroishi, Tominaga et al. 1990; Arai, Kuroishi et al. 1994; Shiomi, Nishiguchi et al. 2001), radionuclide concentration in tumor (Duhaylongsod, Lowe et al. 1995; Shiomi, Nishiguchi et al. 2001), therapeutic effectiveness (Tsunoda, Shibusawa et al. 1992), and histological characteristics of tumor tissue (Lindell, Hartman et al. 2007). Furthermore, the results of all quantitative studies based on changes in growth rate may be different if parameters such as SGR and DT are used. To demonstrate such effects results based on published DT values were compared with those from calculated corresponding SGR values. These examples were selected, because the contradictory results of using SGR and DT could clearly be demonstrated. Otherwise, any set of tumor volume or tumor marker data collected at several time points can be selected to compare the results of quantitative analyses using DT and SGR. In general, the theoretical bases for the difference between DT and SGR are valid for any variable that might be measured with DT or SGR.

SGR is the exponential tumor growth constant, which is the correct measure of tumor growth rate. SGR is least influenced by uncertainties of the measurement procedure and its frequency distribution is symmetric for an exponentially growing tumor, *i.e.* SGR is suitable for common statistical tests. When the growth rate of a tumor is measured with DT, the scale of measurement is nonlinearly transformed from the correct scale of SGR to the incorrect scale of DT (Eq. 6). The SGR of a tumor in clinical observations is in the order of a few tenths %/d to a few %/d. DT does not uniformly indicate the difference between growth rates of tumors throughout all ranges. In addition, DT does not have an absolute zero in its scale and DT is not defined when the growth rate is zero. Furthermore, the frequency distribution of DT (and log DT) is not symmetric, while most of the common statistical tests are based on the assumption of normally distributed variables.

Tumors may follow non-exponential growth model, e.g., the Gompertzian. Non-exponential growth models assume that the growth rate depends on time or size of tumor. The growth model of a non-exponentially growing tumor cannot be explained by a single value, and more parameters are needed to describe the true growth model of each tumor. As a result, the non-exponential growth characteristics of tumors cannot be observed with only two volume measurements. In this work the difference between DT and SGR as quantities of tumor growth rate (at any time point or size) was studied. Therefore, the results are valid whether the growth rate is constant (exponential growth) or varying by time or size of tumor (non-exponential growth). However, non-exponential growth is not usually observed in natural growth of tumors in clinical studies, because the tumors can be followed only for a short time before the start of any treatment.

Assuming that SGR is normally distributed the DT distribution will be positively skewed. This result is expected according to the nonlinear relationship between DT and SGR. It doesn't mean that SGR is normally distributed in clinical observations, but the frequency distribution of SGR can represent the true distribution of growth rates of tumors (whether it is normal or not). Studies have shown that the frequency distribution of DT in clinical observations is positively skewed and the logarithmic transformation of DT is used by some researchers (Spratt and Spratt 1964; Spratt 1969; Shackney, McCormack et al. 1978; Balmukhanov, Turdugulov et al. 1982; Kuroishi, Tominaga et al. 1990; Usuda, Saito et al. 1994). However, the logarithmic transformation cannot fully compensate for the asymmetry of DT distribution. Data on the real frequency distribution of SGR in clinical observations is not available, because DT has been the variable used for quantification of tumor growth rate so far. Furthermore, the true frequency distribution of tumor growth rate is not known in most studies and statistical approaches based on normal distribution of variable are used, e.g., Student's t-test (Satkauskas, Batiuskaite et al. 2005; Lee, Kim et al. 2008). Therefore, the same approach was used to compare groups of tumors both for SGR and DT, regardless of the distribution type of variables. This reveals another aspect of the importance of selecting a proper variable for tumor growth rate. Variations of tumor growth rate due to biological factors or measurement uncertainties are asymmetrically expressed by DT. DT_m can thus correctly estimate the average growth rate of tumors only when the frequency distribution of DT is symmetric, *i.e.*, when the uncertainty of growth rate estimation and the difference between the growth rates of tumors are relatively low. DT_e is the only estimator that can give the true average growth rate of tumors. All measurement results, including negative SGR values, should be included in the calculation of mean SGR. It should be noted that the inclusion of negative values does not mean that such values must exist. Winer-Muram et al. observed the erroneous estimation of the average growth rate with DT_m and made a better estimation by the reciprocal of the average of reciprocals of DT values (Winer-Muram, Jennings et al. 2002). The results of that method are comparable with those using SGR as growth rate parameter.

Differences in growth rates of tumors are mainly a result of different growth fractions, GF (fraction of tumor volume which consists of proliferating cells), and cell loss rates, CLR, *i.e.*, the duration of cell cycle does not play a major role in the varying kinetics of tumor growth (Fingert, Campisi et al. 1993; Rew and Wilson 2000). Such a growth pattern can quantitatively be described using SGR. If the absolute value of CLR is added to the SGR of tumor volume, the SGR of the entire tumor in the absence of cell loss is obtained. The SGR of the proliferating cells can then be obtained by dividing the result by GF. In general, the volumetric SGR of a tumor with polyclonal cell population (a heterogeneous SGR distribution within the tumor) is the mean of SGR values weighted by the fraction of each cell component, including stromal and tumor cell populations, *i.e.*, for a tumor with n cell components: $V=V_1+V_2\ldots+V_n$, then $V\cdot SGR_V=V_1\cdot SGR_1+V_2\cdot SGR_2\ldots+V_n\cdot SGR_n$. Similar to DT_m within a population, calculation of volumetric growth rate of tumor as mean DT of its components will result in erroneous estimations of the growth rates.

Tumor growth and dissemination in clinical studies (Paper III)

The correlation between tumor doubling time and its volume is usually used in clinical studies, where the growth decline with tumor size is to be assessed (Nakamura, Roser et al. 2003; Ozono, Miyao et al. 2004). However, by definition this technique is not mathematically valid according to the Gompertzian growth model. The present study was thus based on the linear relationship between SGR and the logarithm of tumor volume according to the Gompertzian model. Our approach enabled us to estimate metastasis formation times and rates. Akanuma previously attempted to find the model constants for the Gompertzian growth model using the linear correlation between growth rate and the logarithm of tumor volume (Akanuma 1978). His method was based on a graphical estimation of SGR at different tumor volumes. Tumors were scaled according to their doubling time and very high or negative values were excluded.

There are a number of non-exponential growth models available. In this work, only the Gompertzian growth model was evaluated because it is the most commonly adopted model in clinical studies (Bajzer 1999; Afenya and Calderon 2000). The approach is, however, theoretically applicable to any growth model.

The results show that fitting of different growth curves to the volume of each tumor is not sufficient to estimate the true growth model of tumors, when the observation of tumor growth is limited in time, e.g. in clinical observations. In two patients, the growth of metastases was followed for several years, and still the direct curve fitting was insufficient to estimate the true growth model. It is also hazardous to extrapolate growth curves, because different growth models may converge during a short time period and then diverge. Estimations of tumor formation times or metastasis formation rates, based on extrapolations, can be largely

erroneous. The development of more precise quantitative approaches to retrieve all information from available clinical data is therefore crucial. A previous clinical study called for more accurate quantification of the growth rates of human cancer, providing data that is essential for understanding the biological variance of human cancers (Spratt, Meyer et al. 1995). Variances in the observed growth rates of tumors of the same type either in one patient or in a population can be due to: (a) measurement uncertainties, (b) growth deceleration with increasing tumor volume, or (c) other biological differences between tumors. In this work a mathematically more correct approach was developed, compared to conventional methods, to analyze tumor growth in clinical studies. The results showed that for measurement of tumor growth rate at a specific time or volume, SGR is a more appropriate variable than DT, also when the variance was induced by measurement uncertainties (Paper I) or biological factors (Paper II).

In Paper III the relation between SGR and the logarithm of tumor volume was used to assess the growth model of tumors in clinical studies. The growth model of groups of tumors of the same type either in one patient or in a group of patients was examined. A significant correlation between SGR and the logarithm of tumor volume in each group of tumors indicates that growth deceleration is an important factor in variances in the observed growth rates of tumors of the same type either in one patient or in a population. A general Gompertzian growth model might then estimate the growth of all tumors, i.e., the smaller tumors represent the growth of larger tumors when they were of small size and *vice versa*. Lack of correlation between SGR and the logarithm of tumor volume indicates that biological factors other than growth deceleration due to volume are more important in the variances in the observed growth rates of tumors of the same type either in one patient or in a population. These tumors may grow exponentially with different growth rates, or according to the Gompertzian model, but the model constants, SGR_0 and λ, may be distributed heterogeneously among the tumors.

The correlation between SGR and the logarithm of tumor volume was statistically significant regarding the growth of meningiomas in a group of patients from the literature (Nakamura, Roser et al. 2003). Further analysis by dividing the material into small and large tumors also supported this result. A similar growth model observed for a group of patients with one tumor type corroborates that the response rate in this group is a suitable measure to assess the efficacy of novel therapeutics. The result from the second study of meningiomas (Nakasu, Fukami et al. 2005) was different with no significant correlation between SGR and the logarithm of tumor volume.

According to the linear regression of tumor SGR with the logarithm of its volume, the growth of liver metastases in the carcinoid patient were described by a general growth model. This

patient was treated with octreotide. Since, based on curve fitting results, none of tumors in this patient deviate from exponential growth, the treatment was assumed to have no effect on growth of these tumors. The growth of lung metastases in the patient with renal cell carcinoma might biologically differ widely, but were still describable by a general growth model. Analysis of the metastasis formation rate showed that the estimated number of metastases was in line with such an interpretation. Carcinoid liver metastases probably grew according to a general Gompertzian growth model, while renal cancer metastases in the lungs probably grew exponentially with different growth rates. No evidence of non-exponential growth was observed. This result also highlights the need for careful interpretation of model fitting results.

A higher metastasis formation rate in Fig. 9 does not necessarily mean a larger number of metastases at a time point, because the time origin in this figure is the time of the formation of the first metastasis, which is different for different models. A decelerating growth model, *e.g.* the Gompertzian model, implies a higher metastasis formation rate than the exponential model, but the number of metastases should be calculated for any specific time to compare different models. The estimated number of liver metastases in the patient with ileum carcinoid was 9 and 22 at the time of primary surgery using the exponential and the Gompertzian models, respectively. The value of 9 metastases is not realistic, since 24 metastases were imaged beside the studied metastases. The Gompertzian model thus provided the best estimate of the number of liver metastases. The estimated number of lung metastases in the patient with renal cell carcinoma was 14 and 84 at the time of primary surgery according to the exponential and the Gompertzian models, respectively. The value of 84 metastases is probably not realistic, since only one small, non-growing, metastasis was imaged beside the 7 lesions studied. If other metastases were present, they should have grown to visible size during 10 years of follow-up. Here, the exponential model thus provided the best estimate of the number of lung metastases. Our results emphasize the importance of correct information on tumor growth to estimate the number and size distribution of metastases correctly.

Tumor response to therapy (Paper IV)

The presented quantity for objective assessment of solid tumor response to therapy, TR, is a general continuous measure regardless of the mechanism of the effect: cytotoxic and/or cytostatic. Studies have shown that tumor growth rate is a valuable parameter for, e.g., prediction of recurrence after surgery (Cucchetti, Vivarelli et al. 2005) and survival of patients (Blankenberg, Teplitz et al. 1995), and the change in tumor growth rate can serve as a surrogate end-point for determination of therapy response (Haney, Thompson et al. 2001). In this study, a simplified formula was derived based on the effect of therapy on kinetics of tumor growth. Tumor response was measured by the logarithm of the ratio of post-treatment tumor volume to the volume of tumor (at time of efficacy assessment) if therapy was not initiated. TR=0

indicates no effect and larger TR values distinguish more effective therapies from less effective ones. Negative TR value indicates post-treatment tumor swelling or increased growth rate.

The log-ratio (LR) measure for tumor response to therapy, which was used by Kharison et al. (Karrison, Maitland et al. 2007) is calculated as the logarithm of the ratio of post-treatment tumor volume to the pre-treatment tumor volume. The natural growth of tumor between diagnosis and therapy initiation as well as after therapy is neglected in the LR value. There might be a few weeks or longer delay between tumor diagnosis and initiation of therapy due to practical limitations or necessity of further evaluations. Tumors continue to grow during this period. The volume of a tumor with doubling time of 70 days will increase 23% during a three week period. Repopulation of tumor cells during therapy is also an important factor that should not be neglected (Davis and Tannock 2000; Kim and Tannock 2005). TR values are thus larger than corresponding LR values for the same set of tumors. The overall under-estimation of treatment effectiveness by LR (Err) is larger for a rapidly growing tumor or when the time between pre-treatment and post-treatment volume assessments is long. The relative importance of Err also depends on the response effect level obtained, i.e., for a more effective treatment LR is less affected by this error.

The generally used methods when comparing the post-treatment volume of tumor with the pre-treatment volume, e.g. in RECIST, will thus result in underestimation of treatment effectiveness. Analysis of the radionuclide therapy of Non-Hodgkin's lymphoma patients showed that six of eleven patients would be categorized as having progressive disease, according to RECIST, if a pure cytostatic therapy that could completely block tumor growth was initiated. It has already been shown that RECIST underestimates the effect of imatinib on metastatic gastrointestinal stromal tumor (Choi, Charnsangavej et al. 2007). The fact that treatment effectiveness is underestimated by LR or RECIST has important implications on assessing the efficacy of new anticancer drugs or different combinations of therapies.

The fact that clinical trial designs based on LR values are feasible was demonstrated (Karrison, Maitland et al. 2007), which suggests that TR can also be used for such studies. The main difference between TR and LR is the reference volume of tumor for efficacy assessment, which is the volume of corresponding untreated tumor or pre-treatment volume of tumor for TR and LR, respectively. Statistical aspects of using such continuous variables in clinical trials, e.g. handling extreme cases as complete disappearance of lesions are discussed elsewhere (Karrison, Maitland et al. 2007). It is also noteworthy that any natural growth model can be used in the TR calculation (Eq. 17) when determining $V_{n.}$

The major limitation with TR, compared to LR, is the need of knowledge of the natural growth model and growth rate of the tumor. The natural growth rate can in theory be estimated before treatment initiation. In practice, treatment is usually initiated as early as possible after diagnosis. In the present study no such data was available and the growth rate was assumed to be the average re-growth rate of tumors after therapy, which in reality might be clearly different from the true growth rate of tumors before treatment. In this case, these data were used only for demonstration without any clinical interpretation of the results. If tumor volumes at two occasions prior to start of therapy are available, e.g. having two CT scans at diagnostic investigation and an investigation just before therapy initiation, natural SGR of tumor and consecutively V_n can be calculated. Taking both inter- and intra-operator as well as inter-scan variability into account, an increase of the measured volume by more than 25% is needed for a 95% likelihood of being real growth rather than measurement inaccuracy. For an exponentially growing tumor increase of the measured volume will be more than 25% if the measurement time interval between two investigations is longer than 0.32 DT. If tumor volume prior to treatment is only available at one occasion, e.g. the first diagnostic imaging, tumor volume at time of therapy initiation can be estimated by back-extrapolation of volume regression curve during therapy, which might be described by exponential model (Stein, Figg et al. 2008). That measure together with the first tumor volume available can be used for estimation of the natural SGR of tumor.

Tumor structure can be rather non-homogenous consisting of, e.g., different clones of cancer cells (with different sensitivities to an anticancer agent), stroma, and necrotic areas. The value of SGR of a tumor may thus be obtained from the spatial distribution of SGR values within the tumor:

$$\mathrm{SGR} = \frac{1}{\mathrm{V}} \oint_{\mathrm{V}} \mathrm{sgr}(\mathrm{x}, \mathrm{y}, \mathrm{z}) \cdot \mathrm{dx} \cdot \mathrm{dy} \cdot \mathrm{dz} \,, \qquad (20)$$

where sgr(x,y,z) is for each part of tumor and SGR is for tumor volume. An effective treatment can reduce sgr(x,y,z) differently in different parts of the tumor depending on, e.g., pharmacokinetics and dose response of a systemically used agent. This will accordingly cause a reduction in SGR of tumor as was used in the presented model (Eq. D). Studies have shown that functional imaging variables might be correlated with tumor growth rate, e.g. using PET (Duhaylongsod, Lowe et al. 1995; Tann, Sandrasegaran et al. 2008). Further developments in this field may facilitate tumor SGR estimation by a functional imaging before treatment and also integration of TR based anatomical changes in tumor into other means of tumor response assessment by functional imaging with MRI (Chenevert, McKeever et al. 1997) or PET (Stroobants, Goeminne et al. 2003; Boss, Olmos et al. 2008).

Conclusions

Quantification of the volumetric growth rate of tumor using doubling time can give very different results compared to using the specific growth rate, which is a correct quantity for tumor growth rate. The average growth rate of tumors must also be estimated by the mean SGR or its equivalent doubling time. The uncertainty of SGR can be reduced with increasing measurement time interval, or decreasing volume measurement uncertainties. In addition, SGR is a suitable parameter for common statistical testing based on the assumption of normally distributed parameters. This conclusion is also valid for determination of the increase rate of tumor marker level, whether it is correlated with the growth rate of tumor volume or not.

With limited measurements available in clinical studies fitting of different growth curves to the data is not sufficient to estimate the true growth model of tumors. Analysis of the correlation between growth rate and the volume of tumor may give better estimate of tumor growth model for some types of tumors. Formation times and formation rates of metastases may also be estimated by the linear regression of SGR with the logarithm of tumor volume.

Available criteria for assessment of tumor response to therapy, including RECIST, neglect natural growth rate of tumor, which leads to underestimation of treatment effectiveness. Logarithm of the ratio between treated tumor volume and the volume of corresponding non-treated tumor is a general continuous quantity for tumor response, useful for both cytostatic and cytotoxic treatments. The concept may also accommodate integration of anatomical changes of tumor into changes in other biological characteristics of tumor after therapy.

Acknowledgements

This study was supported by grants from the Swedish Cancer Society, the Assar Gabrielsson Foundation, Göteborg, Sweden, and the King Gustav V Jubilee Clinic Cancer Research Foundation, Göteborg, Sweden.

I would like to express my gratitude to Dr. Peter Bernhardt, my supervisor, and Prof. Eva Forssell-Aronsson, my co-supervisor, for giving me this opportunity. I appreciate your support and being always encouraging throughout my work.

I also appreciate the contribution of my co-authors, Prof. Håkan Ahlman, Dr. Viktor Johanson, and Dr. Lars Kölby, who have participated in this work in different ways. I would like to acknowledge Prof. Ragnar Hultborn and Dr. Zakaria Einbeigi for important discussions on clinical aspects of this study, and Dr. Magnus Båth and Prof. George Sgouros for valuable communications.

Thanks to my roommates and all other colleagues at the Department of Radiation Physics for making the department a nice work place.

I would like to express my especial gratitude to my parents. My father taught me analytical thinking long before I started school. I am most grateful for his influence on the title of my thesis! My mother, my best friend, has always been there for me with her support. And finally, thanks to Sahar for her assistance during my work.

I dedicate this work to the love of my life, my son, Arya. I love you!

Further studies

This work can be categorized as translational research, where basic theory is directly applied to patient related data. It ended up with a new framework for quantitative studies in clinical settings rather than conclusive interpretations based on the presented clinical data. The results will hopefully lead to enhanced use of frequency distributions of SGR, and studies of correlation between SGR and different variables, e.g., different tumor markers levels or tumor volume, based on larger number of patient data to facilitate decisive interpretations in clinical studies. However, for future formulations based on SGR, it must be noted that SGR is a mathematical concept that can be virtually calculated and the absolute relative growth rate of tumor approaches SGR when the measurement time interval approaches zero. While %/d is appropriate for tumor growth rate in clinical settings, %/h or %/min are more appropriate for more rapidly growing tumors, e.g., in animal models or cell culture, respectively.

An interesting feature of SGR is its capability for future multiscale modelings, where data on, e.g., cellular heterogeneities within tumor quantified with, e.g., functional imaging (SPECT, PET or MRI), can be linked to volumetric changes of tumor. Anatomical and functional means of evaluation of tumor response to therapy can also be integrated within such models.

Tumor response, TR, measure formulated in this work can be used for future clinical trials on novel, or combinations of, anticancer therapeutic modalities. Accurate measure of TR facilitates a more correct evaluation of therapeutic efficacy in correlation with other variables, e.g., tumor size or growth rate and patient survival.

TR is the measure of the effect of therapy on natural growth of tumor. However, the same approach can be used for evaluation of the effect of any other factor that may influence the tumor growth e.g., the function of the immune system.

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