Clinical and Translational Radiation Oncology 10 (2018) 36–41

Contents lists available at ScienceDirect

Clinical and Translational Radiation Oncology

journal homepage: www.elsevier.com/locate/ctro

Short Communication

MATLAB®-based fitting method to evaluate survival fractions after multimodal treatment

Jolanta Marzec [a], Lukasz Marzec [b], Peter Martus [c], Daniel Zips [a], Arndt-Christian Müller [a,*]

[a] Department of Radiation Oncology, Eberhard Karls University, Hoppe-Seyler-Strasse 3, 76072 Tübingen, Germany
[b] Zentrum für Datenverarbeitung, Eberhard Karls University, Wächterstraße 76, 72074 Tübingen, Germany
[c] Institute for Clinical Epidemiology and Applied Biometry, Eberhard Karls University, Silcherstraße 5, 72076 Tübingen, Germany

ARTICLE INFO

Article history:
Received 11 March 2018
Accepted 27 March 2018
Available online 29 March 2018

Keywords:
Survival fraction
MATLAB®-based fitting method
Multimodal treatment
LQ-Model

ABSTRACT

To easily analyse and visualize cell kill dynamics measured by survival fraction after single or combined treatments a MATLAB®-based application was developed. A statistical analysis with different options of visualisation of single and combined treatment effects can be performed in a few steps not requiring advanced knowledge of statistical programs.

Introduction

Clonogenic assays are used to quantify cellular response to anticancer agents including radiation [1]. The steepness of the initial slope of the survival curve correlates with clinical responsiveness [2,3]. Therefore, clonogenic assays are frequently used tests in radiation research [4,5]. However, the graphical and statistical evaluation of multimodal treatments is challenging because the mode of interaction of response modifiers and irradiation as well as the used radiation fraction size might limit the applicability of the LQ-model.

To analyse the effect of irradiation plus an additional modality also becomes tedious because a normalization of data points has to be performed for each treatment [6]. Employment of statistic software for evaluation is possible but programming of survival curve evaluation tools might become relatively complex and time consuming for a radiation oncologist or a radiobiologist as illustrated elsewhere [7].

Therefore, we developed a standalone GUI (graphical user interface)-application to analyse radiosensitivity measured by survival fraction after single and combined treatments in vitro. The LQ-Script was created in MATLAB®, which is a programming language available in many academic and research institutions providing reliable and proven algorithms and their implementation in GUIs [8]. The MATLAB®-based GUI application and their capabilities are demonstrated to give researchers of radiobiology an insight and easy access to relevant information on this script. Furthermore, this report can be utilized as an instruction, how to analyse combined treatments using survival fractions by this MATLAB®-based LQ-Script.

Methods

MATLAB® program

A MATLAB®-based program was developed to estimate α/β-parameters and to visualize approximated surviving fraction (SF) curves. System requirements were MATLAB® 2010 or newer. The linear quadratic (LQ)-Model [1,9–11] was used to calculate cell kill kinetics after irradiation. The Eqs. (1) and (2) describe the LQ regression with error term (±δ i.e. mean zero and homoscedastic variance σ^2):

$$SF = \exp - (\alpha D + \beta D^2) \pm \delta \quad (1)$$

D is the radiation dose in Gy. On a log-linear plot of the survival curve, α is the cell kill per Gy of the linear component and β is the cell kill per Gy^2 of the quadratic component. In assumption that in case of combined treatment the drug has it owns effect the following equation was applied:

* Corresponding author.
E-mail addresses: jolanta.marzec@med.uni-tuebingen.de (J. Marzec), lukasz.marzec@uni-tuebingen.de (L. Marzec), peter.martus@med.uni-tuebingen.de (P. Martus), daniel.zips@med.uni-tuebingen.de (D. Zips), arndt-christian.mueller@med.uni-tuebingen.de (A.-C. Müller).

https://doi.org/10.1016/j.ctro.2018.03.003
2405-6308/

$$SF = SF0 * \exp - (\alpha D + \beta D^2) \pm \delta \quad (2)$$

Plating efficacy (PE) was calculated according to the following equation [7]:

$$PE = \text{number of colonies counted/number of cells plated} \quad (3)$$

Survival fraction (SF) was calculated according to the following equation

$$SF = \text{PE of treated sample/PE of control} \quad (4)$$

The script uses fit functions of MATLAB® including confidence intervals and prediction bounds. The algorithm is described elsewhere (http://uk.mathworks.com/help/curvefit/fit-postprocessing.html). The confidence interval and prediction bounds can be selected. Level of certainty is 0.95 (=95%). For users who have installed the "Statistics and Machine Learning Toolbox" additional options are available including standard errors for the estimated coefficients α, β, and SF0 along with t-statistics and p-values. The coefficient of determination (R^2) is calculated as a goodness-of-fit measure and overall significance is measured by F-statistics. Both are given as output statistics. For advanced users, we integrated a feature allowing for plotting residuals of the fitted models. It is named "plot residuals".

Data set

As example for combined treatment, we utilized a modified colorectal cancer cell line of HCT-116 which was cultivated as described elsewhere [12]. After graded radiation doses (0Gy, 2 Gy, 3.5 Gy or 5 Gy) with and without simultaneous in vitro cytotoxic drug (incubation with topoisomerase inhibitor type I) the colony forming assay was performed in delayed plating technique [13]. The plating efficiency (PE) and surviving fraction (SF) were calculated by the script as described above.

Script installation

The Supplement contains the Zip-File "Matlab_based_script.zip". Please, unzip the file to a separate folder. The following five files are included:

LQFit.m – the MATLAB®-based LQ-Script
SampleData.mat – a MATLAB® sample data file
Main.Fig. and Main.m – graphical user interface of the script
InitLQFitStruct.m – a script facilitating data structure input

Results

A MATLAB® based script named "LQ-Script" was developed enabling visualization and statistical analysis of combined treatment effects. The instruction (development of data source structure, workflow and an example file of LQ-Script were attached (Supplement).

Algorithms

Two fitting methods are available. The first one is the classical linear fitting method, implemented as a build-in MATLAB® function named fit called with the parameter Method set to LinearLeastSquares. This method uses the linear least squares method optionally with a robust parameter (bisquare) to provide LQ-model approximation. The second one is the nonlinear least squares curve fitting method implemented as the same build-in MATLAB® function fit, called with parameter Method set to NonLinearLeastSquares.

Both methods use as default the unmodified MATLAB® implementation of the Levenberg–Marquardt algorithm [14,15]. Optionally the Trust-Region algorithm could be used. The script sets the following options to run the nonlinear least squares method, Table 1.

Curve visualization

A MATLAB®-based fit was approximated for an exemplary data set of combined irradiation and cytotoxic drug in vitro. The fit-curves were approximated und visualized on the basis of surviving fractions after irradiation. The number of data points which can be evaluated is not restricted.

In the "Main" window, the following evaluation tools can be selected (Fig. 1A): At first, the cell line(s) and drug concentrations are chosen for the evaluation by using "String" and a single mouse click (i.e. the sample "Some cell line" is chosen and the two drug concentrations 0 and 2.5 are selected). The next step is to define the regression type (linear/nonlinear, see above). Three different **LQ-analyses** are available:

The first option "LQ-Analysis normalized by the control" can be used to derive LQ-curves with normalization to control (i.e. 0 Gy) with and without a drug (Fig. 1A and A) to evaluate log-*additive effects*. The second (classical) option "LQ-Analysis normalized by SF0 [Gy]". In this analysis the normalization to SF0 = 1 is performed as typically used for analysis of survival fractions and can be applied for evaluation of *supra*-log-*additivity* for instance if a response modifier is used (Fig. 1A and B). The third option "LQ-Analysis" equals LQ analysis without normalization to visualize the *distribution of raw data* (Fig. 1A and C).

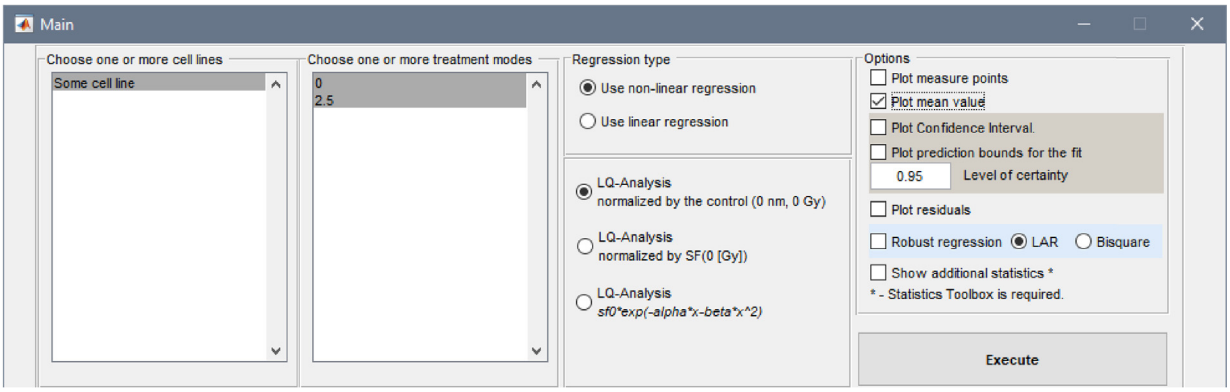
The following display options are available and refer to colony counts. Options include "Plot mean value", "Plot Confidence Interval" (CI), "Plot residuals". The degree of certainty (usually 0.95) can be manually adapted for CI as well as for prediction bounds. "Plot measure points" and "Plot prediction bounds" (PB) are only available for "LQ-Analysis" (raw data). The robust regression can be selected either as "LAR" (least absolute residual method) or as Bisquare. The corresponding R^2 is calculated and shown in the command window (Fig. 1B). The best fit (R near 1) should be selected. In addition, α, β, SF0 and goodness of fit are calculated and displayed. Using the box "show additional statistics" the F statistics, mean, standard error (SE), t-statistics, p-values are shown. Furthermore, confidence interval (CI), survival (%), Plating efficacy (PE in%) and CI of PE are calculated and displayed (Fig. 1C).

All permutations of fitcurves can be visualised (in our example irradiation alone, combined treatment at each radiation or chemotherapeutic dose). A 95%-confidence interval (CI) was

Table 1
Initial conditions for the Levenberg–Marquardt algorithm. The initial guess of the parameter has to be provided to start running the nonlinear least squares method.

Parameters of the nonlinear least squares method	Item/value
Initial values for the coefficients	α:0 β:0 sf0:1
Algorithm	Levenberg-Marquardt
Maximum change in coefficients for finite difference gradients	0.1
Minimum change in coefficients for finite difference gradients	10e−8
Maximum number of evaluations of model allowed	600
Maximum number of iterations allowed for fit	400
Termination tolerance on model value	10e−6
Termination tolerance on coefficient values	10e−6

(A)



(B)

```
Command Window
>> load('SampleData.mat')
>> Main
-------------- Some cell line - 0 --------------
Nonlinear regression model using the fit method provided by the Curve Fitting Toolbox.
Note: The model below will be used for plot operations.
Some cell line - 0
     General model:
     y(x) = sf0*exp(-alpha*x-beta*x^2)
     Coefficients (with 95% confidence bounds):
       alpha =     0.04937  (-0.07116, 0.1699)
       beta =      0.1029  (0.06054, 0.1452)
       sf0 =       100.1  (94.81, 105.4)
Goodness of fit.
          sse: 3.6317e+04
       rsquare: 0.8346
           dfe: 141
    adjrsquare: 0.8323
         rmse: 16.0489
```

(C)

```
****
* Nonlinear regression model using the fitnlm method provided by the Statistics and Machine Learning Toolbox.
* Note: The model below will NOT be used for plot operations. It provides additional statitics regarding estimated coefficients.
****

Nonlinear regression model:
    y ~ (b1*exp( - b2*x - b3*x^2))

Estimated Coefficients:
          Estimate       SE        tStat       pValue
          ________    ________    ______    __________

    b1      100.09      2.6736     37.438    3.9338e-75
    b2    0.049348     0.06097    0.80939       0.41966
    b3     0.10289    0.021416     4.8043    3.9246e-06


Number of observations: 144, Error degrees of freedom: 141
Root Mean Squared Error: 16
R-Squared: 0.835,  Adjusted R-Squared 0.832
F-statistic vs. zero model: 663, p-value = 6.67e-83
b1 - SF0
b2 - alpha
b3 - beta
----
Level of certainty used to calculate Confidence Intervals (CIs_SF, CIs_PE): 95%.
          Mean_SF    StdErr          CIs_SF          Survival      PE          CIs_PE
          _______    _______    ________________    ________    _____    _______________

    0         100     3.4694    '63.3456, 77.4322'      100      12.5    '1.7553, 15.8419'
    2      60.695     1.0286    '40.634, 44.8105'     60.695    7.5868    '3.252, 7.4285'
    3.5    22.139    0.92099    '13.7136, 17.453'     22.139    2.7674    '0.078217, 3.8176'
    5      8.4057    0.45316    '4.9967, 6.8366'      8.4057    1.0507    '-0.18039, 1.6596'

fx >>
```

Fig. 1. Curve visualization and statistics. A) "Main" window shows selectable evaluation tools. B) The calculated statistics is displayed in the "Command window". C) In case of choosing "show additional statistics" the calculations are also displayed in the "Command window", too.

chosen to graphically compare data, Fig. 2. Furthermore, the diagrams can be customized by a built-in Graph Editor with export options to different image file formats (e.g. .JPG or .PNG).

Combined treatment i.e. combined irradiation and cytotoxic drug can be demonstrated in two different modes to measure additivity and supra-additivity. Additivity is defined as the sum of both single effects of two modalities i.e. addition of a drug to irradiation results in a parallel shift of the survival curve. Supra-additivity is defined as an effect being larger than the addition of single effects of two modalities [16]:

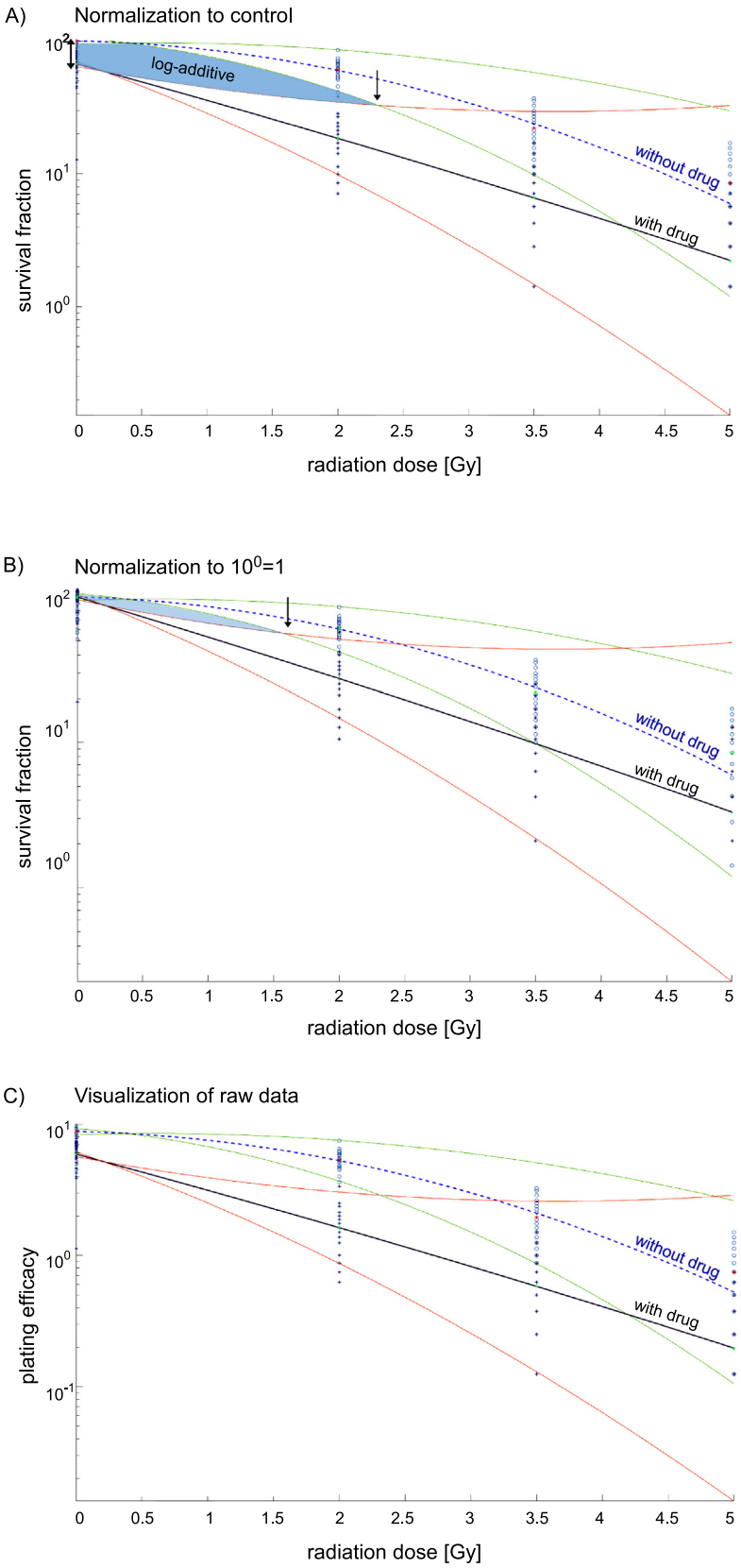


Fig. 2. Visualization of log-additive, supra-log-additive effects and raw data. A) Normalization to each control (i.e. with and without drug): The difference at 0 Gy between both curves demonstrates the log-additive effect of a drug and is indicated by an arrow. A significant log-additive effect is indicated by the dark blue area until the crossing (second arrow) of the confidence interval of both curves (red/green CIs). B) Normalization to $10^0 = 1$ (classical analysis of radiosensitivity without a drug): A significant supra-log-additive effect is measured until crossing of both CIs of the radiation response curves with and without drug in a semi-logarithmic scale. In the example, the CI (red/green CIs) cross before the first applied radiation dose at ~1.5 Gy (indicated by the blue area). Therefore, no supra-log-additive effect was measured since no radiation dose has been tested between 0 and 1.5 Gy. C) Raw data can also be visualized in a semi-logarithmic scale (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

a) Normalization to each control (i.e. with and without drug): Survival fractions were normalized to each control (the **"log"-additive effect** of combined modalities was visualized in a semi-logarithmic scale). Heterogeneous response to multimodal treatment was visualized using the option to start the combined treatment curve at the level of cell kill related to the second modality (i.e. drug dose levels) at 0 Gy. A significant additive effect is indicated by the dark blue area until the crossing (arrow at ~ 2.4 Gy) of the confidence intervals (CI) of both curves, Fig. 2A.
b) Normalization to 10^0=1 (classical analysis of radiosensitivity without a drug or to show supra-log-additive effects of a drug): The combined modality curve was normalized to 10^0=1 in order to visualize a potential radio-sensitizing (**supra-log-additive**) effect of the cytotoxic drug depending on concentration of chemotherapy. A potential supra-log-additive effect is measured until crossing of both CIs in a semi-logarithmic scale, Fig. 2B. In the example, the CIs (red/-green) cross before the first applied radiation dose at ~1.5 Gy (indicated by the blue area and the arrow). Therefore, no supra-log-additive effect was measured since no radiation dose has been tested between 0 and 1.5 Gy.
c) No normalization can be chosen to visualize raw data in a semi-logarithmic scale, Fig. 2C.

Taken together, log-additive and supra-log-additive effects (using different normalization methods, Fig. 2) can easily be graphically visualized and distinguished with this application.

Discussion

We developed a new software tool (LQ-Script) dedicated for visualizing combined treatment effects. All needed parameters are point-by-point requested from the user ensuring a comprehensive data visualization. Furthermore, a script for structure level definition is available to avoid challenging programming on the command line [7]. The user does not need to decide which parameters are needed, which coefficients should be used nor the analysis model. We demonstrated the functionality of the script. The resulting diagram enables a graphical comparison of curves in regard to statistical significance by evaluating confidence intervals and prediction bounds. Moreover, statistical features like F-statistics and goodness of fit were implemented. Supra-additivity as well as additivity of combined treatment can be evaluated by two modes of normalization. The α/β-value can be calculated allowing to annualize the effect of different fractionation schedules.

However, the possibility to present data on the basis of the LQ-algorithm should not lead to the assumption that combined treatment (i.e. radiation treatment and a response modifier) always follow the LQ-mode. The LQ-model can safely be applied to hyper-fractionated [17,18] and conventional fractionated radiation schedules [19,20]. There are some lines of evidence that the LQ-model can also cautiously be used for moderate and extreme hypofractionation including stereotactic body radiotherapy (SBRT) [21,22]. However, criticism exists due to the not regarded impact of radioresistant subpopulations [23] and underestimation of isoeffective doses of normal tissue with large fraction sizes (>9 Gy) [24]. The applicability of the LQ-model can also be affected depending on mechanism of action of a response modifier and depending on culture conditions [25]. Depending on the choice of radiation dose range, another challenge could occur. In case of a strong radiosensitizing effect and radiation doses close to a potential threshold of the LQ-model, the threshold of the LQ-model could also be exceeded.

In summary, this MATLAB®-based script offers features enabling a graphical evaluation of surviving curves and permits to investigate the influence of radiation response modifiers. However, limitations of the LQ-model and modes of interaction have to be taken into account to carefully evaluate combined treatment effects. In addition, MATLAB@ is a software usually available at academic sites. However, biannual updates of MATLAB@ could lead optical changes of the LQ-Script.

Conclusion

An easy to use standalone MATLAB®-based GUI-application has been created visualizing single and combined treatment effects. The developed software resolves current limitations of other available templates of spreadsheet analysis programs like restrictions concerning total number of evaluated cells, combined treatment effects and comparison of different sample sizes. A complex data analysis can be conveniently performed by the MATLAB®-script. Graphical comparisons of different data sets become much easier. The wide range of options (presentation of all measure points and the mean, 95% CI, PB) facilitates data analysis. The clearly arranged, expandable input data structure can be used to perform some additional data processing. The software is available in the Supplement.

Conflict of interest

None.

Authors' contributions

JM has made substantial contributions to conception, design, and discussion of the software script and writing of the manuscript. LM has developed the MATLAB®-based script and made substantial contributions to writing of the manuscript. PM has tested the MATLAB®-based script and made substantial contributions to evaluation of statistical tools and application of algorithms i.e. statistics. DZ was involved in revising the manuscript for important intellectual content and gave final approval of the version to be published. ACM has made substantial contributions to conception, design, and discussion of the software script and writing of the manuscript.

All authors read and approved the final manuscript.

Acknowledgment

We thank Prof. Stephan Huber for discussion and very helpful counselling regarding essential tools of the script needed for laboratory utilization. We acknowledge support by Deutsche Forschungsgemeinschaft and Open Access Publishing Fund of University of Tübingen.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ctro.2018.03.003.

References

[1] Hall EJ, Giaccia AJ. Radiobiology for the Radiologist. Lippincott Williams & Wilkins; 2006.
[2] Deacon J, Peckham MJ, Steel GG. The radioresponsiveness of human tumours and the initial slope of the cell survival curve. Radiotherapy Oncol: J Eur Soc Therapeutic Radiol Oncol 1984;2(4):317–23.
[3] Malaise EP, Fertil B, Chavaudra N, Guichard M. Distribution of radiation sensitivities for human tumor cells of specific histological types: comparison of in vitro to in vivo data. Int J Radiat Oncol Biol Phys 1986;12(4):617–24.

[4] Chen JL, Chen JP, Huang YS, Tsai YC, Tsai MH, Jaw FS, Cheng JC, Kuo SH, Shieh MJ. Radiosensitization in esophageal squamous cell carcinoma: effect of polo-like kinase 1 inhibition. Strahlentherapie und Onkologie: Organ der Deutschen Rontgengesellschaft [et al.] 2016;192(4):260–8.
[5] Hashimoto S, Sugie C, Iwata H, Ogino H, Omachi C, Yasui K, Mizoe JE, Shibamoto Y: Recovery from sublethal damage and potentially lethal damage: Proton beam irradiation vs. Xray irradiation. Strahlentherapie und Onkologie: Organ der Deutschen Rontgengesellschaft [et al.], 2017.
[6] Venkatachalam S, Mettler E, Fottner C, Miederer M, Kaina B, Weber MM. The impact of the IGF-1 system of cancer cells on radiation response 2013; An in vitro study. Clin Transl Radiation Oncol 2017;7:1–8.
[7] Franken NA, Rodermond HM, Stap J, Haveman J, van Bree C. Clonogenic assay of cells in vitro. Nat Protoc 2006;1(5):2315–9.
[8] Creating Apps with Graphical User Interfaces in MATLAB [http://de.mathworks.com/discovery/matlab-gui.html].
[9] Barendsen GW. Parameters of linear-quadratic radiation dose-effect relationships: dependence on LET and mechanisms of reproductive cell death. Int J Radiat Biol 1997;71(6):649–55.
[10] Fowler JF. The first James Kirk memorial lecture. What next in fractionated radiotherapy? British J Cancer Suppl 1984;6:285–300.
[11] Kellerer AM, Rossi HH. The biophysical properties of 3.9-GeV nitrogen ions. VI. Interpretation of results. Radiat Res 1973;55(3):447–56.
[12] Li M, Jung A, Ganswindt U, Marini P, Friedl A, Daniel PT, Lauber K, Jendrossek V, Belka C. Aurora kinase inhibitor ZM447439 induces apoptosis via mitochondrial pathways. Biochem Pharmacol 2010;79(2):122–9.
[13] Marzec J: Radiosensibilisierung durch CPT-11 und pro-apoptotische Mechanismen bei der Radiochemotherapie im kolorektalen Zellmodell. In. Tübingen: Medizinische Fakultät; 2014.
[14] Gavin HP. The Levenberg-Marquardt method for nonlinear least squares curve-fitting problems 2013:1–17.
[15] Least-Squares (Model Fitting) Algorithms, Levenberg-Marquardt Method [http://uk.mathworks.com/help/optim/ug/least-squares-model-fitting-algorithms.html#f204].
[16] Schmoll HJ, Höffken K, Possinger K: Kompendium Internistische Onkologie Standards in Diagnostik und Therapie: Springer-Verlag, Berlin Heidelberg; 2006.
[17] Baumann M, Herrmann T, Koch R, Matthiessen W, Appold S, Wahlers B, Kepka L, Marschke G, Feltl D, Fietkau R, et al. Final results of the randomized phase III CHARTWEL-trial (ARO 97–1) comparing hyperfractionated-accelerated versus conventionally fractionated radiotherapy in non-small cell lung cancer (NSCLC). Radiotherapy Oncol: J Eur Soc Therapeutic Radiol Oncol 2011;100(1):76–85.
[18] Bentzen SM, Saunders MI, Dische S. From CHART to CHARTWEL in non-small cell lung cancer: clinical radiobiological modelling of the expected change in outcome. Clin Oncol (R Coll Radiol) 2002;14(5):372–81.
[19] Fowler JF. The linear-quadratic formula and progress in fractionated radiotherapy. Br J Radiol 1989;62(740):679–94.
[20] Thames HD, Bentzen SM, Turesson I, Overgaard M, Van den Bogaert W. Time-dose factors in radiotherapy: a review of the human data. Radiotherapy Oncol: J Eur Soc Therapeutic Radiol Oncol 1990;19(3):219–35.
[21] Brenner DJ. The linear-quadratic model is an appropriate methodology for determining isoeffective doses at large doses per fraction. Seminars Radiation Oncol 2008;18(4):234–9.
[22] Brown JM, Carlson DJ, Brenner DJ. The tumor radiobiology of SRS and SBRT: are more than the 5 Rs involved? Int J Radiat Oncol Biol Phys 2014;88(2):254–62.
[23] Kirkpatrick JP, Meyer JJ, Marks LB. The linear-quadratic model is inappropriate to model high dose per fraction effects in radiosurgery. Seminars Radiation Oncol 2008;18(4):240–3.
[24] Sheu T, Molkentine J, Transtrum MK, Buchholz TA, Withers HR, Thames HD, Mason KA. Use of the LQ model with large fraction sizes results in underestimation of isoeffect doses. Radiotherapy Oncol: J Eur Soc Therapeutic Radiol Oncol 2013;109(1):21–5.
[25] Franken NA, Oei AL, Kok HP, Rodermond HM, Sminia P, Crezee J, Stalpers LJ, Barendsen GW. Cell survival and radiosensitisation: modulation of the linear and quadratic parameters of the LQ model (Review). Int J Oncol 2013;42(5):1501–15.