Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/rpor

Review

# Radiobiology of stereotactic body radiation therapy (SBRT)

*Miquel Macià i Garau*

*Radiation Oncology Department and Translational Research Laboratory, Institut Català d'Oncologia (ICO), L'Hospitalet de Llobregat, Catalonia, Spain*

ARTICLE INFO

*Article history:*
Received 3 February 2016
Received in revised form
19 January 2017
Accepted 26 February 2017
Available online 23 March 2017

*Keywords:*
Radiobiology
Stereotactic body radiation therapy
SBRT

ABSTRACT

Recent advances in the technology of radiotherapy have enabled the development of new therapeutic modalities that deliver radiation with very high accuracy, reduced margins and high dose conformation, allowing the reduction of healthy tissue irradiated and therefore minimizing the risk of toxicity. The next step was to increase the total tumor dose using conventional fractionation (which remains the best way to relatively radioprotect healthy tissues when large volumes are treated) or to use new fractionation schemes with greater biological effectiveness. Based on the experience gained in radiosurgery, the latter way was chosen for small and well-defined tumors in the body. Stereotactic body radiotherapy delivers high doses of radiation to small and well-defined targets in an extreme hypofractionated (and accelerated) scheme with a very high biological effectiveness obtaining very good initial clinical results in terms of local tumor control and acceptable rate of late complications. In fact, we realize a posteriori that it was not feasible to administer such biologically equivalent dose in a conventional fractionation because the treatment could last several months. So far, these new therapeutic modalities have been developed due to technologic advances in image guidance and treatment delivery but without a solid biological basis. It is the role of traditional radiobiology (and molecular radiobiology) to explain the effects of high doses of ionizing radiation on tumor and normal tissues. Only through a better understanding of how high doses of ionizing radiation act, clinicians will know exactly what we do, allowing us in the future to refine our treatments. This article attempts to describe through simple and understandable concepts the known aspects of the biological action of high doses of radiation on tumor and normal tissues, but it is clear that we need much more basic research to better understand the biology of high doses of radiation.



## 1. Background

Stereotactic body radiation therapy is a new radiation treatment method to deliver, with high accuracy, a high dose of radiation to small and well-defined targets, utilizing either a single dose or a few fractions with a high degree of precision within the body.[1] This new high technology modality requires a high degree of precision, accuracy and reproducibility of the entire treatment delivery process. Maneuvers to limit the

E-mail address: mmacia@iconcologia.net
http://dx.doi.org/10.1016/j.rpor.2017.02.010

movement of the target volume and stereotactic localization of the lesion or image guidance are mandatory for target localization, minimization of margins and dose delivery. Patient immobilization systems should be very accurate and radiation equipment should have mechanical tolerances for radiation delivery of ±2 mm. In order to minimize normal tissue toxicity, conformation of high doses to the target and rapid dose fall-off gradients away from the target is performed using multiple coplanar, non-coplanar static fields or arc therapy. It must be emphasized that quality assurance is a critical step of the entire process.[2]

Initial clinical experiences with SBRT show impressive results in terms of local tumor control and acceptable late complications rate. This high rate of local tumor control, not fully explained by our current understanding of the response of tumors to high doses of radiation, raises the question of whether there is some new biology to explain these results.[3] Although, from a biological point of view, the major feature that differentiates SBRT from conventional radiation treatment is the delivery of large doses in one or a few fractions which results in a high biological effective dose, the radiobiology of SBRT is poorly understood. Other important radiobiological distinctive features of SBRT are small irradiated volumes, inhomogeneous dose distribution and short overall treatment time. The classic basic principles of fractionation, namely the Withers four R's of radiotherapy (repair, repopulation, redistribution and reoxygenation)[4] and intrinsic radiosensitivity cannot be totally ignored in explaining the effects of high doses of ionizing radiation on tumors and normal tissues but new radiobiological knowledge on the biological effects of high doses of ionizing radiation is emerging allowing us in the future to better understand how high doses of ionizing radiation act and refine SBRT treatments.

## 2. Classic biological basis of high-dose radiotherapy on tumors

### 2.1. *Tumor radiosensitivity*

Radiosensitivity is the susceptibility of cells (tissues and organs) to be damaged and inactivated by ionizing radiation. To compare the radiosensitivity of different types of cells, we can use parameters directly read on the cell survival curve as the surviving fraction at 2 Gy ($SF_2$) or parameters derived from mathematical models. The linear-quadratic (LQ) formalism is the most commonly used tool to compare fractionation sensitivity, to model the effect of fractionation in conventionally fractionated radiotherapy and to predict tumor response to altered fractionation regimens. The model is based on the assumption that cell death is due to DNA strand breaks. However, studies have shown that the LQ model overestimates cell killing at high single doses because it predicts a survival curve that continuously bends downward whereas the experimental data are consistent with a constant slope at high doses.[5,6] Therefore, there is concern that LQ model does not accurately predict tumor cell response at the higher doses per fraction used in SBRT. In fact, there is a controversy about the limitations of the LQ model for predicting the biological effectiveness of SBRT. Proponents of the use of the model argue that it is a mechanistic, biologically based model related to single and double-strand DNA breaks; it has sufficiently few parameters to be practical; it has well-documented predictive properties for fractionation/dose-rate effects in the laboratory and it is reasonably well validated, experimentally and theoretically, up to about 10 Gy/fraction and would be reasonable for use up to about 18 Gy per fraction.[7] However, other authors believe that the use of the LQ model is inappropriate because much of the data used to generate the model are obtained in vitro at doses well below those used in SBRT and does not consider the impact of radiation on cells other than the tumor cells (for example, the indirect tumor cell death caused by vascular damage); it does not accurately explain the observed clinical data and ignore the impact of radioresistant subpopulations of cells.[8] In any case, literature of SBRT is full of examples where BED (biologically effective dose) or EQD2 (equivalent dose in 2 Gy fractions) are used to compare different fractionations.[8–11,24] The only parameter required to perform the calculations is the $\alpha/\beta$ ratio. Although there are many uncertainties, the $\alpha/\beta$ ratio normally used for early-responding tissues and most tumors is $\geq$10 Gy. No need to say that the choice of this parameter is critical to the correct calculations of BED or EQD2. For instance, some slow-growing tumors such as prostate cancer, breast cancer, melanoma and soft tissue sarcomas have $\alpha/\beta$ ratios well below 10 Gy. Two examples of how to perform the calculations are shown below.

1. Assuming full reoxygenation and complete repair of sublethal damage between fractions and no repopulation, calculate the BED and EQD2 of a treatment that delivers 54.00 Gy in 3 fractions of 18.00 Gy to a lung tumor (estimated $\alpha/\beta$ ratio = 10.00 Gy).

$$\mathbf{BED_{(\alpha/\beta)} = D \cdot (1 + d/\alpha/\beta)}$$

$$BED_{10} = 54 \cdot (1 + 18/10) = 151.20\ Gy_{10}$$

$$\mathbf{EQD2 = BED_{(\alpha/\beta)} = D \cdot (1 + 2/\alpha/\beta)}$$

$$EQD2 = 151.20 = D \cdot (1 + 2/10) = 126\ Gy$$

Therefore, using the simplest formula of LQ model, 54.00 Gy delivered in 3 fractions to a lung tumor is equivalent to 126 Gy delivered in 63 fractions of 2 Gy for an estimated $\alpha/\beta$ ratio of 10.00 Gy.

2. Assuming full reoxygenation and complete repair of sublethal damage between fractions and no repopulation, calculate the BED and EQD2 of a treatment that delivers 36.25 Gy in 5 fractions of 7.25 Gy to a prostate cancer (estimated $\alpha/\beta$ ratio = 1.5 Gy).

$$\mathbf{BED_{(\alpha/\beta)} = D \cdot (1 + d/\alpha/\beta)}$$

$$BED_{1.5} = 36.25 \cdot (1 + 7.25/1.5) = 211.46\ Gy_{1.5}$$

$$\mathbf{EQD2 = BED_{(\alpha/\beta)} = D \cdot (1 + 2/\alpha/\beta)}$$

$$EQD2 = 211.46 = D \cdot (1 + 2/1.5) = 90.62\ Gy$$

Therefore, using the simplest formula of LQ model, 36.25 Gy delivered in 5 fractions to a prostate cancer is equivalent to 90.62 Gy delivered in 45 fractions of 2 Gy for an estimated $\alpha/\beta$ ratio of 1.50 Gy.

**Table 1 – Tumor control probability as a function of isocenter $BED_{8.6}$, EQD2, $BED_{USC}$, and SED (values read directly on the curves 1 and 2 in Mehta publication).[12]**

| TCP (%) | BED ($Gy_{8.6}$) | EQD2 (Gy) | $BED_{USC}$ ($Gy_{USC}$) | SED (Gy) |
|---|---|---|---|---|
| 50 | 61 | 50 | 47 | 35 |
| 60 | 75 | 65 | 60 | 50 |
| 70 | 105 | 86 | 75 | 65 |
| 80 | 125 | 95 | 95 | 87 |
| 90 | 159 | 125 | 124 | 128 |

For early-stage non-small cell lung cancer (NSCLC), clinical data suggest that radiobiological modeling using the LQ formula is adequate to explain the efficacy of SBRT. Recently, Mehta et al. reviewed the available clinical tumor control (≥2 years) probability (TCP) data for SBRT (single dose and 3–8 multifractions) and 3-dimensional conformal radiation therapy (3D-CRT) to determine the relationship between TCP and BED in medically inoperable stage I NSCLC.[12] They found a sigmoidal relationship between TCP as a function of BED with TCP ≥ 90% achieved with BED ≥ 159 $Gy_{8.6}$ and EQD2 ≥ 125 Gy (Table 1) (Figures 1 and 2 in Mehta publication). Replotted data distinguishing between single fraction SBRT, multifraction SBRT and 3D-CRT showed a monotonic relationship between TCP and BED for SBRT and 3D-CRT data (Figure 1 in Brown editorial) suggesting that SBRT and 3D-CRT produce the same TCP probability when adjusted for BED.[13] The authors concluded that at least for early NSCLC, the high rate of local tumor control achieved by SBRT can be fully explained by the much higher biological effective dose and that there is no need to invoke a "new biology" to explain these results.[13,14]

A new radiobiological model called universal survival curve (USC) to compare different fractionations of both conventionally fractionated radiotherapy and SBRT was published in 2008.[15] This model was constructed to provide a superior approximation of the experimentally measured survival curve data in the high-dose range by hybridizing the LQ model survival curve for the low-dose range (the shoulder) and the multitarget model asymptote for high-dose range. According to the USC model, $D_T$ is the transition dose from LQ model to the multitarget model. From this hybrid model, two equivalent functions can be derived: the biologically effective dose and the single fraction equivalent dose (SFED) for both conventionally fractionated radiotherapy and SBRT. BED is calculated by the LQ formula if dose per fraction is below the transition dose $D_T$ and by USC formula if dose per fraction is higher than $D_T$. SFED is defined as the dose delivered in one fraction that has the same biological effect as the tested dose-fractionation scheme. Again, two different USC formulas should be used depending on whether the dose per fraction is lower or higher than $D_T$. Equating the two formulas allows to calculate the Standard Effective Dose (SED) that is the total dose administered in 2 Gy fractions for the same effect.

The validity of this new model was tested using previously published radiosensitivity parameters of human lung cancer cell lines. The model fits very well with a cell survival curve of H460 NSCLC obtained by clonogenic assay.[15] The main weakness of the model is its dependence on five radiobiological parameters: $\alpha$, $\beta$, $D_0$, $D_q$ and $D_T$. Two examples of calculations are shown below.

1. Calculation the $BED_{USC}$, SFED and SED of a treatment that delivers 54.00 Gy in 3 fractions of 18.00 Gy to a lung tumor (estimated $\alpha = 0.33\ Gy^{-1}$; $D_0 = 1.25$ Gy; $D_q = 1.80$ Gy; $D_T = 6.2$ Gy and $\alpha/\beta$ ratio = 10.00 Gy).

$$\mathbf{BED_{USC}} = \frac{1}{(\alpha \cdot D_0)} \cdot (D - n \cdot D_q)$$

$$\mathrm{BED_{USC}} = \frac{1}{(0.33 \cdot 1.25)} \cdot (54 - 3 \cdot 1.80) = 2.42 \cdot 48.6 = 117.6\ Gy_{USC}$$

$$\mathbf{SFED} = D - (n - 1) \cdot D_q$$

$$\mathrm{SFED} = 54 - (3 - 1) \cdot 1.8 = 54 - 3.60 = 50.40\ Gy$$

$$\mathbf{SED} = \frac{1}{(\alpha \cdot D_0)} \cdot \frac{D_{sbrt} - n_{sbrt} \cdot D_q}{(1 + 2/\alpha/\beta)}$$

$$\mathrm{SED} = \frac{1}{(0.33 \cdot 1.25)} \cdot \frac{54 - 3 \cdot 1.80}{(1 + 2/10)} = 2.42 \cdot 40.50 = 98.01\ Gy$$

Therefore, using the formulas of USC model, 54.00 Gy delivered in 3 fractions of 18.00 Gy (>$D_T$) to a lung tumor results in a SFED of 50.40 Gy that is equivalent to 98.01 Gy delivered in 49 fractions of 2 Gy for an estimated $\alpha = 0.33\ Gy^{-1}$; $D_0 = 1.25$ Gy; $D_q = 1.80$ Gy and $\alpha/\beta$ ratio = 10.00 Gy.

2. Calculation using the Universal Survival Model if 33 Gy in 3 fractions and 37 Gy in 5 fractions are equipotent fractionations (estimated $\alpha = 0.33\ Gy^{-1}$; $D_0 = 1.25$ Gy; $D_q = 1.80$ Gy; $D_T = 6.2$ Gy and $\alpha/\beta$ ratio = 10.00 Gy).

$$\mathbf{BED_{USC}} = \frac{1}{(\alpha \cdot D_0)} \cdot (D - n \cdot D_q)$$

$$\mathrm{BED_{USC}} = \frac{1}{(0.33 \cdot 1.25)} \cdot (33 - 3 \cdot 1.80) = 2.42 \cdot 27.6 = 66.8\ Gy_{USC}$$

$$\mathrm{BED_{USC}} = \frac{1}{(0.33 \cdot 1.25)} \cdot (37 - 5 \cdot 1.80) = 2.42 \cdot 28 = 67.8\ Gy_{USC}$$

$$\mathbf{S_{FED}} = D - (n - 1) \cdot D_q$$

$$\mathrm{SFED} = 33 - (3 - 1) \cdot 1.8 = 33 - 3.6 = 29.40\ Gy$$

$$\mathrm{SFED} = 37 - (5 - 1) \cdot 1.8 = 37 - 7.2 = 29.80\ Gy$$

$$\mathbf{SED} = \frac{\mathbf{1}}{(\boldsymbol{\alpha} \cdot \boldsymbol{D_0})} \cdot \frac{\boldsymbol{D_{sbrt}} - \boldsymbol{n_{sbrt}} \cdot \boldsymbol{D_q}}{(\mathbf{1} + \mathbf{2}/\boldsymbol{\alpha}/\boldsymbol{\beta})}$$

$$\mathrm{SED} = \frac{1}{(0.33 \cdot 1.25)} \cdot \frac{33 - 3 \cdot 1.80}{(1 + 2/10)} = 2.42 \cdot 23 = 55.66\,\mathrm{Gy}$$

$$\mathrm{SED} = \frac{1}{(0.33 \cdot 1.25)} \cdot \frac{37 - 5 \cdot 1.80}{(1 + 2/10)} = 2.42 \cdot 23.33 = 55.45\,\mathrm{Gy}$$

Therefore, using the formulas of USC model, 33 Gy in 3 fractions and 37 Gy in 5 fractions are equipotent fractionations for an estimated $\alpha = 0.33\,\mathrm{Gy}^{-1}$; $D_0 = 1.25$ Gy; $D_q = 1.80$ Gy; $D_T = 6.2$ Gy and $\alpha/\beta$ ratio = 10.00 Gy.

In the study cited above for early-stage NSCLC treated with single fraction SBRT, multifraction SBRT and 3D-CRT, TCP as a function of $\mathrm{BED_{USC}}$ calculated by USC formulas was also sigmoidal with TCP ≥ 90% achieved with $\mathrm{BED_{USC}} \geq 124\,\mathrm{Gy_{USC}}$ and SED ≥ 128 Gy ($\alpha = 0.33\,\mathrm{Gy}^{-1}$; $D_0 = 1.25$ Gy; $D_q = 1.80$ Gy; $D_T = 6.0$ Gy and $\alpha/\beta$ ratio = 8.6 Gy) (Table 1) (Figures 1 and 2 in Mehta publication).[12]

As expected, the curves relating TCP and BED calculated by LQ model or by USC model were different but with minimal clinical significance for high dose per fraction used in SBRT.

Although the high rate of local tumor control achieved by SBRT for early NSCLC could be fully explained by the high biological effective dose calculated by the LQ model or by the USC model, this may not be true for other types of tumors. Emerging evidence indicates that apart from direct tumor cell killing, SBRT can severely damage the tumor neovasculature leading to indirect tumor cell death due to an acute decrease in blood perfusion. If this were true, none of the two models described above would take into account this effect[8] (see Section 4).

For liver metastases, a wide variety of SBRT dose and fractionation schedules have been reported. Fractionation schemes used in prospective phase I–II trials range from single fraction of 14–30 Gy [15,17] to hypofractionated schedules of 30–75 Gy [18–22,38] delivered in 3–5 fractions in an overall treatment time between 5–6 days and 2.5 weeks. One risk stratified phase I trial used doses between 27.70 Gy and 60 Gy delivered in 6 fractions over 2 weeks.[23] Published reports are difficult to compare not only because of heterogeneity in dose-fractionation regimens but also due to the heterogeneity in dosimetric planning and dose prescription, heterogeneity in patient selection, primary tumor, number and volume of liver tumors, number of systemic treatments before and after SBRT and heterogeneity in the definition of local control. Overall, these phase I-II trials report local control rates ranging from 56% to 100% at 2 years. Higher doses are associated with better local control but the dose response curve for local control is uncertain. In a pooled analysis of patients with colorectal liver metastases treated by SBRT at 3 institutions, Chang et al. found in multivariate analysis that total dose, dose per fraction and BED all correlated with local control. The estimated $\mathrm{BED_{10\,Gy}}$ needed for a 90% local control at 1 year was 117 $\mathrm{Gy_{10}}$. Converting this value into a 3-fraction SBRT regimen using the LQ-formula yields an estimated total dose between 46 and 52 Gy to achieve 90% local control.[24] The same calculation for a 5-fraction regimen results in a total dose around 55 Gy. Based on this publication, a total prescription dose of 48 Gy or higher in 3 fractions is recommended when possible.[24] Better outcome has been reported for non-colorectal liver metastases. This is perhaps because most patients with colorectal liver metastases had been heavily pretreated before SBRT.[25] In conclusion, there is not a consensus on the standard approach to the SBRT for liver oligometastases and the appropriate dose and fractionation scheme remains undefined. New trials of SBRT for liver metastases are needed to clarify the dose-fractionation local control relationship.

### 2.2. *Repopulation of tumor cells*

Repopulation of tumors cells refers to the proliferation of the surviving clonogenic tumor cells during the course of a fractionated radiotherapy. Once started, radiation therapy (and chemotherapy) can trigger surviving clonogenic tumors cells to divide faster than before, a phenomenon known as accelerated repopulation or rapid compensatory repopulation. It is thought that accelerated repopulation is the cause of the detrimental effect on local control of the prolongation of the overall treatment time and the basis for accelerated radiotherapy in rapidly proliferating tumors. Withers was the first to describe this phenomenon and showed that clonogen repopulation in squamous cell carcinomas of the head and neck accelerates after a lag period of 4 ± 1 weeks after initiation of radiotherapy.[26] $T_k$ (kick-off) is the elapsed time between the start of fractionated radiotherapy and the onset of accelerated repopulation in tumors (or rapid renewing tissues). $T_k$ has been estimated for some tumors: 3–4 weeks for head and neck tumors,[26,27] 19 days for cervical cancer[28] and between 30 and 69 days, depending on the stage, for prostate cancer.[29]

Although nobody really knows the onset time of accelerated repopulation of tumors treated with SBRT, data derived from conventionally fractionated radiotherapy suggest that this phenomenon is probably not very important for SBRT given the relatively short overall treatment time (and irrelevant in single dose SBRT). Therefore, reducing overall treatment time to around one or two weeks as in fractionated SBRT may be advantageous in terms of reducing the possibility of accelerated tumor repopulation in particular for rapidly proliferating tumors and may contribute to the improvement of local control.[30] A practical consequence is that the mathematical repopulation term should not be added to the simple formula if the LQ model is used to calculate equivalences.

In spite of the above, a multi-institutional retrospective study of 505 lung tumors treated with SBRT with a variety of fractionation regimens surprisingly showed that shorter treatment duration was significantly associated with a better local control. Patients who received SBRT over ≤10 elapsed days had a 2 year local recurrence of 4% versus 14% for ≥11 days ($p < 0.01$).[10]

### 2.3. *Reoxygenation of tumors cells*

Due to the imbalance between the growth of tumor cells and vasculature, many rapidly proliferating tumors have hypoxic areas because tumor cells are far from the vessels and therefore are deprived of oxygen and nutrients. In addition, newly formed vessels are abnormal, immature,

chaotically distributed and functionally deficient causing areas of acute or chronic hypoxia and necrosis. It is well known that hypoxic tumor cells are 2–3 times more radioresistant than well oxygenated tumor cells and this may be the cause of the lack of response to radiotherapy or tumor recurrences.[31] Reoxygenation, a phenomenon that only occurs in tumor tissues, is the process by which the surviving hypoxic tumor cells become better oxygenated during the period after the initiation of a fractionated irradiation and therefore more radiosensitive to the following radiation doses.[32] The biological mechanisms involved in the phenomenon of reoxygenation are poorly understood but are probably different for acute and chronic hypoxia. Reoxygenation can occur quickly in the case of acute or transient decreased perfusion caused by an intermittent blood flow through an abnormal and immature vessel. On the contrary, reoxygenation of areas with chronic hypoxia may take several hours or days to resolve, which is believed to be due to tumor shrinkage after delayed mitotic death and reabsorption of cellular debris. Therefore, reducing the overall treatment time reduces a chance that reoxygenation of chronic hypoxic tumor areas may occur. Although tumor shrinkage is probably faster when using SBRT because many tumor cells are killed and die more rapidly due to other modes of radiation-induced cell death different from delayed mitotic death and some extent of reoxygenation may occur, the short overall treatment time plays against the reoxygenation of hypoxic tumor areas, particularly in larger tumors presumably less oxygenated. Using this theoretical reasoning, some authors advocate leaving inter-fraction intervals $\geq$72 h to facilitate reoxygenation.[33,45]

Needless to mention that for single-fraction SBRT, reoxygenation is an irrelevant process and therefore the existence of radioresistant hypoxic tumor cells constitutes a potential problem. A recently published phase II study conducted by RTOG comparing two different SBRT schedules for patients with medically inoperable stage I peripheral NSCLC showed a primary tumor control at 1 year of 97% for a single fraction SBRT of 34 Gy versus 92.7% for a multifraction SBRT of 48 Gy delivered in 4 consecutive daily fractions (along with a lower rate of toxicities).[34] This and other studies with longer follow-up (local control at 2- and 3-years of 95% and 88.1% in Indiana University Phase II trial [35,36] and estimated 3- and 5-year primary tumor control rate of 97.6% and 93% in RTOG 0236 phase II trial using 54 Gy in 3 fractions of 18 Gy in 1.5–2 weeks[37,38]) seem to show that high doses of radiation would be able to overcome the problem of tumor hypoxia radioresistance of lung tumors. Although some local failures can be explained by geographical errors, local tumor control decreases over time especially in larger tumors. Some authors, using single doses of SBRT, have shown differences in local tumor control depending on tumor volume, attributing these differences to radioresistance mechanisms associated with tumor hypoxia and proposing to test the addition of hypoxic cell radiosensitizers to a single dose of SBRT.[39,45]

In metastatic sites treated with multifraction SBRT, local tumor control also seems related to the size of lesions suggesting that there may be mechanisms of radioresistance perhaps related to the existence of hypoxic areas in larger tumors.[40–42]

### 2.4. *Redistribution of tumor cells in the cell cycle*

Radiosensitivity of cells varies depending on the cell cycle phase in which they are at the time of irradiation, being more radioresistant in the S-phase. Redistribution is the process by which, after transient cell cycle arrest due to the activation of cell cycle checkpoints by radiation, the surviving tumor cells become more sensitive to radiation because they progress through the cell cycle to more radiosensitive phases. Although the biological significance of this phenomenon, if any, is unknown in SBRT, shortening the overall treatment time plays against redistribution of tumor cells into more radiosensitive phases of the cell cycle. In an in vitro study on human promyelocitic leukemic cells, high single dose of irradiation indiscriminately caused cell cycle arrest and interphase death in all cell cycle phases.[43]

### 2.5. *Repair of sublethal damage of tumor cells*

The existence of molecular mechanisms of DNA damage repair allows cells to repair radiation sublethal damage before the next radiation fraction and thus increase cell survival (recovery) as a function of time between fractions or during treatment delivery. Cells need time to sense and repair radiation-induced DNA damage. Although the kinetics of repair of sublethal damage probably involves at least two components, a quick and slow repair phases, it is believed that rapidly proliferating tumors have, like early-responding normal tissues (and unlike late-responding normal tissues), short sublethal damage repair half-times. Therefore, there will be no residual unrepaired damage in rapidly proliferating tumors cells after irradiation at high doses in 24 h intervals fractionation.

On the other hand, there may be some repair of sublethal damage during irradiation if the exposure time is lengthened. New treatment techniques and SBRT administer the dose in a more prolonged fraction delivery time allowing the repair of some sublethal damage in rapidly proliferating tumor cells during the treatment and therefore decreasing the biological effect.[44] It has been estimated that any fraction delivery that lasts more than half an hour can cause a clinical significant loss of biological effect.[45,46] As clinical data are lacking, it is strongly recommended for the purpose of future studies on biological efficacy to record and report the duration of overall fraction time, especially if fraction delivery time is prolonged more than half an hour.[46]

## 3. Classic biological basis of high-dose radiotherapy on healthy tissues and organs

The advantages of high doses of radiation to the tumor tissues in terms of local control must be balanced against the risk of complications mainly dependent of late-responding tissues. Normal tissue dose tolerance depends on fraction size, total dose, time between fractions, overall treatment time, volume of normal tissue or organ irradiated, type of organ and other factors (host factors: genetic susceptibility, age, comorbidities and therapeutic factors: concomitant therapies, previous treatments including previous radiotherapy).

In order to minimize the risk of toxicity, it is imperative to minimize the volume of healthy tissue irradiated using proper clinical indications (small and well-defined targets not embedded inside a functionally organized serial organ), and reducing PTV margins using modern and accurate technology, good patient immobilization, effective maneuvers to limit target movements, stereotactic target localization or image guidance and a good technique with conformation of high doses to the target and rapid fall-off doses away from the target.

### 3.1. *Radiosensitivity of late-responding tissues and organs*

All the doubts expressed in Section 2.1. regarding the use of the LQ model must also be considered in its application to the prediction of late effects. Due to the nature of the tissue, normal late-reacting tissues (with low $\alpha/\beta$ ratio) are more radiosensitive at higher doses per fraction than rapidly proliferating tumors and early-reacting normal tissues. Therefore, and even taking into account the normal tissue volume effect (better tolerance at lower volume), the risk of late complications is higher at higher doses per fraction. Models of normal tissue complication probability (NTCP) should be used with caution because they are imperfect and should be validated with more mature data on late effects collected in the long-term monitoring of well conducted SBRT clinical trials.

One important question is how organs at risk are functionally organized. Depending on how the functional subunits are organized, organs can be divided into two categories. In parallel functioning organs (lung, liver), the different functional subunits are structurally well defined and perform the same function. They are usually large organs and as their functional subunits operate separately, they show redundancy in their function and organ reserve (surgeons can remove part of the organ). Serially functioning organs (spinal cord, airways) have structurally undefined functional subunits that must work together to maintain organ function. They are usually long organs acting as a conduit and if a part of the organ is seriously damaged then all the downstream function is lost (surgeons cannot remove any part of the organ).[47,48] This is the reason why the most important determinant of serially functioning organ toxicity is the maximum dose of radiation.[35,49–52] This is also the reason why the treatment of a tumor embedded within a serially functioning organ is not a good indication of SBRT. On the other hand, parallel functioning organs can tolerate localized high doses of radiation if a significant volume of the organ can be avoided and so can continue to maintain its function. Therefore, the most important determinant of parallel functioning organ toxicity is the volume of the organ irradiated and its basal functional status (comorbidities).[41,51–53]

#### 3.1.1. *Dose-volume constraints for organs at risk*

The report of the American Association of Physicists in Medicine Task Group 101 states that "normal tissue dose limits for SBRT are considerably different from conventional radiotherapy due to extreme dose-fractionation schemes and are still quite immature".[2] Actually, most dose-volume constraints are based on retrospective studies or simply using theoretical calculations based on the LQ model. Therefore, the dose limits should be used with care and should be continually reviewed and validated pending more mature data on late effects collected in the long-term monitoring of well conducted SBRT clinical trials. For example, the initial dose constraint for normal liver (a minimum volume of 700 mL should receive a total dose of less than 15 Gy in 3 fractions) used in the study of Rusthoven for liver metastases[41] was derived using BED calculations ($\alpha/\beta = 3.00$ Gy) from published experiences in hepatic accelerated hyperfractionation.[57] This and other phase I trials have confirmed the validity of this dose-volume constraint or have described new limitations for single fraction or five fractions SBRT of hepatic metastases.[17,21] The phase I study of Rule established the safety of delivering 60 Gy in 5 fractions to treat liver metastases if pretreatment hepatic function was adequate and a critical volume of 700 mL of normal liver receives a total dose of no more than 21 Gy in 5 fractions.[21] The situation is completely different for the treatment of hepatocellular carcinoma because associated cirrhosis causes a significant decrease in liver tolerance to radiation.[52] It should also pay special attention to centrally located hepatic lesions where the central biliary system is a serially functioning organ.[58,59]

In conclusion, a prudent approach, is to use dose-volume constraints for organs at risk summarized in updated guidelines[2,54] or determined in high-quality phase I trials.[16,21,23,35,41,52,55,56]

### 3.2. *Repopulation and redistribution of late-responding tissues*

Although they could play a role in opposite directions (repopulation increasing the proliferation of normal cells and redistribution increasing normal cell kill) neither repopulation nor redistribution play an important role in the normal tissue response to high doses of ionizing radiation due to the nature of late-reacting tissues with slow cell turnover and long cell lifespan.

### 3.3. *Repair of sublethal damage of late-responding tissues*

Incomplete repair can be a problem for some late-responding normal tissues if large doses are administered without enough interfraction time to allow for complete repair of sublethal damage. It is known that the kinetics of repair of sublethal damage of late-responding normal tissues is slower than that of early-responding normal tissues with longer sublethal damage repair half-times, in the order of several hours.[60] Therefore if large dose fractions create more sublethal damage than conventional fractions and it takes longer time to be repaired, there will be residual unrepaired damage if interfraction time is too short to allow for complete repair. If a significant amount of residual unrepaired damage remains after a too short interfraction time, the accumulation of residual damage to the damage produced by the subsequent fraction can result in an excess of toxicity. A possible example illustrating this phenomenon is shown in the prospective phase II clinical trial of King et al. using SBRT for low-risk prostate cancer.[61] They compared late urinary and rectal toxicities reported by patients using validated quality of life

questionnaires between a SBRT schedule of 36.25 Gy in 5 fractions in five consecutive days with those treated with the same dose administered every other day. While late urinary toxicity showed only a tendency toward improvement with more protracted treatment (19% versus 5%), late moderate or severe rectal toxicity showed a statistically significant difference also in favor of the longer treatment (38% versus 0%; $p = 0.0035$).

To reduce the risk of toxicity due to the accumulation of unrepaired residual damage and since there are no conclusive biological or clinical data, a prudent approach is to perform multifraction SBRT with interfraction times greater than 24 h (remember that tumor repopulation does not seem to be a problem if the overall treatment time is not extended too much).

## 4. Radiation-induced vascular damage in tumors

As well as the direct effect of tumor cell killing caused by double strand breaks in DNA (and modelized in LQ formalism), ionizing radiation can cause damage to other cellular components of tumor microenvironment that may lead to secondary or indirect effects.

Recently, Park et al. reviewed the studies on radiation-induced vascular changes in human and experimental tumors reported in the literature.[62] They concluded that while the functional vascularity in human tumors remains unchanged or improves slightly during the early period of conventional fractionated radiotherapy but gradually diminishes during the later part of treatment, irradiation with doses higher than 10 Gy in a single fraction or 20–60 Gy in limited numbers of fractions causes severe vascular damage leading to indirect death of tumor cells due to the acute decrease in blood perfusion making the tumor environment hypoxic, acidic and deprived of nutrients.

Although these observations are derived from studies with experimental tumors, they hypothesized that similar vascular damage would occur in human tumors irradiated with high-dose hypofractionated radiotherapy. This theory is supported by other experimental data. For instance, Garcia-Barros et al. demonstrated that the exposure of transplanted mouse fibrosarcoma and melanoma cell lines to single dose of 15–20 Gy was followed by a rapid wave of endothelial cell apoptosis soon after irradiation which in turn was followed by death of tumor cells at 2–3 days.[63] Apoptosis in endothelial cells after high doses of radiation could be induced via the acid sphingomyelinase mediated generation of ceramide, which is not activated with doses used in conventional fractionated radiotherapy.[64] Although this phenomenon could be highly variable depending on fraction size, total dose of radiation, location and tumor type, there are insufficient data to ensure that it plays an important role in the effects of high-dose radiation on all tumors.[14] In fact, there is much controversy in the literature about the existence of indirect cell death by devascularization and, if proved true, how this indirect effect can be modeled because it is not taken into account in the original LQ model.[3,8,13,14,64–66] A recent paper, published by Kim reviews experimental data demonstrating the contribution of indirect cell death secondary to vascular damage to total tumor cell death.[67] On the contrary, Moding et al. using dual recombinase technology, generated primary sarcomas in a genetically engineered mouse model with targeted genetic mutations specifically in tumor cells or endothelial cells and demonstrated that tumor cells, rather than endothelial cells, are critical targets that regulate sarcoma eradication by radiation therapy.[68]

An additional hypothesis, linked to the previous theory, to explain the good clinical results of SBRT is the possibility that high doses of radiation can damage the perivascular niche where radioresistant stem cells are living.[62]

## 5. Radiation-induced immunologic effects

It is not the purpose of this section to summarize the complex world of cancer immunology and the complex interactions between ionizing radiation and tumor microenvironment, including immune cells, but only to introduce some concepts.

Radiotherapy has often been seen as an immunosuppressive agent, but today we know that in certain circumstances, radiation may have immunostimulatory effects. A new form of radiation-induced cell death, called immunogenic cell death (ICD), has been recently described.[69,70] ICD is characterized by a massive release of cancer cell neoantigens and the generation of molecular signals (DAMPS: damage-associated molecular patterns) produced by dying cells acting as “eat-me” signals promoting uptake and presentation of tumor-derived antigens by dendritic cells. Remember that activation of natural antitumor T cell response requires uptake and cross-presentation of tumor-derived antigens by dendritic cells to T cells in the draining lymph nodes. Radiation also induces the secretion of interferon I necessary to recruit and activate dendritic cells. This type of cell death not only induced by ionizing radiation can be expressed by tumor cells depending, among others, on tumor type, genetic alterations, immunogenicity but also on fraction size and total radiation dose. Lee et al. observed in an animal model that a dose of 15–25 Gy in one fraction resulted in a dramatical increase in T-cell priming in draining lymphoid tissues leading to the reduction or eradication of the primary tumor or distant metastasis in a $CD8^+$ T cell-dependent fashion and that this phenomenon was not seen with conventional fractionation.[71] $CD8^+$ cells are the major effector cytotoxic T cells.

Radiation has also effects facilitating the trafficking and homing of effector T cells to tumors by the induction of chemokines and endothelial expression of vascular adhesion molecules that facilitate the extravasation of $CD8^+$ T cell into the tumor. In addition, radiation induces upregulation of major histocompatibility class I molecules and death receptors improving the recognition of tumor cells by cytotoxic $CD8^+$ T cells. In short, radiation can induce a massive release of cancer cell neoantigens to the immune system and can facilitate the circulation, recognition and killing of tumor cells by effector T cells. All these immunostimulatory effects of radiation are faced by immunosuppressive effects of the microenvironment or produced by radiation itself. The presence in the tumor microenvironment of cells suppressing antitumor immunity, such as myeloid-derived suppressor cells or regulatory T cells, are examples of the former. Radiation induced

enhancement of tumor infiltration by myeloid-derived suppressor cells or killing of radiosensitive effector T cells by a protracted radiation therapy are examples of immunosuppressive effects of radiation.

Ways to enhance immunostimulatory effects of radiation (use of high dose fractions in short overall treatment time) and/or to overcome immunosuppressive effects of microenvironment (combination of radiation with immunodrugs) are under active investigation.

### 5.1. *Radiation induced abscopal effects*

There is emerging evidence that radiation therapy not only acts locally but can also exert distant non-targeted or systemic effects.[72] Also known as distant bystander or out-of-field effects, the term abscopal effect which is derived from the Latin prefix ab- "position away from" and -scopos "mark or target for shooting" was proposed to describe a tumor event occurring at a distance from the irradiated volume but within the same organism.[73] Clinical reports of an abscopal effect after radiation therapy are few, it is observed anectodally and probably constitutes a clinically under-recognized and under-reported phenomena. Abscopal effects have been described after conventional radiation therapy and more recently after ablative radiation therapy.[74] Two mechanistic explanations have been proposed to account for the abscopal effect: the induction of cytokines, eliciting augmented tumor surveillance, tumor growth inhibition and tumoricidal effects and/or the activation of the immune system.[75] Evidence in experimental models suggests that the abscopal effect is tumor specific and is in part immune mediated and that T cells are required to mediate distant tumor inhibition induced by radiation.[76]

On the other hand, there is an emerging hallmark of cancer: the ability of tumor cells to evade immune destruction.[77] According to the theory of immune surveillance that propose the existence of immunological mechanisms on constant alert against the appearance of transformed cells, the growth of malignant tumors results from development of evasion mechanisms of immune surveillance by neoplastic cells and/or mechanisms of limitation of the attack of the immune system effector cells.[78] Thus, there is a renewed interest in immunotherapy not only in finding stimulating factors of antitumor immunity but also in the study of tumor immune evasion mechanisms and pharmacological countermeasures.

For both reasons, there is an emerging interest in combining radiation therapy and immunotherapy. Recent examples of objective clinical responses achieved by the combination of radiotherapy and immunotherapy support the view that both treatments can act synergistically.[79,80] The combination of radiation therapy and immunotherapy is under active preclinical and clinical investigation. In the USA, more than 50 clinical trials are currently testing the addition of radiation therapy to various immunotherapies.[81,82] Strong efforts should be made to identify the correct dose, fractionation, target volumes and sequencing with the best immunotherapy agent. As a new role for radiation therapy is emerging as a radiation-driven immunotherapy, four extremely important final considerations should be made for our Radiation Oncology community. We need to refine our knowledge of basic and cancer immunology to continue basic research in radiobiology labs, specially in relation to the immune system (immunogenic cell death). We need also to enlarge our knowledge of new immunotherapy drugs (anti CTLA4, anti PD1, ...) and to participate in clinical research not only recruiting patients but also designing clinical trials.

## Conflict of interest

None declared.

## Financial disclosure

None declared.

## REFERENCES

1. Potters L, Steinberg M, Rose C, et al. American Society for Therapeutic Radiology and Oncology and American College of Radiology. Practice guideline for the performance of stereotactic body radiation therapy. *Int J Radiat Oncol Biol Phys* 2004;**60**(4):1026–32.
2. Benedict SH, Yenice KM, Followill D, et al. Stereotactic body radiation therapy: the report of AAPM Task Group 101. *Med Phys* 2010;**37**(8):4078–101.
3. Brown JM, Koong AC. High-dose single-fraction radiotherapy: exploiting a new biology? *Int J Radiat Oncol Biol Phys* 2008;**71**(2):324–5.
4. Withers HR. The four R's of radiotherapy. *Adv Radiat Biol* 1975;**5**:241–7.
5. Gilbert CW, Hendry JH, Major D. The approximation in the formulation for survival $S = \exp - (\alpha D + \beta D^2)$. *Int J Radiat Biol* 1980;**37**(4):469–71.
6. Garcia LM, Leblanc J, Wilkins D, Raaphorst GP. Fitting the linear-quadratic model to detailed data sets for different dose ranges. *Phys Med Biol* 2006;**51**(11):2813–23.
7. Brenner DJ. The linear-quadratic model is an appropriate methodology for determining isoeffective doses at large doses per fraction. *Semin Radiat Oncol* 2008;**18**(4):234–9.
8. Kirkpatrick JP, Meyer JJ, Marks LB. The linear-quadratic model is inappropriate to model high dose per fraction effects in radiosurgery. *Semin Radiat Oncol* 2008;**18**(4):240–3.
9. Stepans KL, Djemil T, Reddy CA, et al. A comparison of two stereotactic body radiation fractionation schedules for medically inoperable stage I non-small cell lung cancer. *J Thorac Oncol* 2009;**4**(8):976–82.
10. Kestin L, Grills I, Guckenberger M, et al. Dose–response relationship with clinical outcome for lung stereotactic body radiotherapy (SBRT) delivered via online image guidance. *Radiother Oncol* 2014;**110**(3):499–504.
11. Koshy M, Malik R, Weichselbaum RR, Sher DJ. Increasing radiation therapy dose is associated with improved survival in patients undergoing stereotactic body radiation therapy for stage I non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys* 2015;**91**(2):344–50.
12. Mehta N, King CR, Agazaryan N, et al. Stereotactic body radiation therapy and 3-dimensional conformal radiotherapy for stage I non-small cell lung cancer: a pooled analysis of biological equivalent dose and local control. *Pract Radiat Oncol* 2012;**2**(4):288–95.
13. Brown JM, Brenner DJ, Carlson DJ. Dose escalation, not "new biology", can account for the efficacy of stereotactic body

radiation therapy with non-small cell lung cancer. *Int J Radiat Oncol Biol Phys* 2013;**85**(5):1159–60.
14. 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.
15. Park C, Papiez L, Zhang S, et al. Universal survival curve and single fraction equivalent dose: useful tools in understanding potency of ablative radiotherapy. *Int J Radiat Oncol Biol Phys* 2008;**70**(3):847–52.
16. Herfarth KK, Debus J, Lohr F, et al. Stereotactic single-dose radiation therapy of liver tumors: results of a phase I/II trial. *J Clin Oncol* 2001;**19**(1):164–70.
17. Goodman KA, Wiegner EA, Maturen KE, et al. Dose-escalation study of single-fraction stereotactic body radiotherapy for liver malignancies. *Int J Radiat Oncol Biol Phys* 2010;**78**(2):486–93.
18. Mendez-Romero A, Wunderink W, Hussain SM, et al. Stereotactic body radiation therapy for primary and metastatic liver tumors: a single institution phase I–II study. *Acta Oncol* 2006;**45**(7):831–7.
19. Hoyer M, Roed H, Hansen AT, et al. Phase II study on stereotactic body radiotherapy of colorectal metastases. *Acta Oncol* 2006;**45**(7):823–30.
20. Ambrosino G, Polistina F, Costantin, et al. Image-guided robotic stereotactic radiosurgery for unresectable liver metastases: preliminary results. *Anticancer Res* 2009;**29**(8):3381–4.
21. Rule W, Timmerman R, Tong I, et al. Phase I dose-escalation study of stereotactic body radiotherapy in patients with hepatic metastases. *Ann Surg Oncol* 2011;**18**(4):1081–7.
22. Scorsetti M, Arcangeli S, Tozzi A, et al. Is stereotactic body radiation therapy an attractive option for unresectable liver metastases? A preliminary report from a phase 2 trial. *Int J Radiat Oncol Biol Phys* 2013;**86**(2):336–42.
23. Lee MT, Kim JJ, Dinniwell R, et al. Phase I study of individualized stereotactic body radiotherapy of liver metastases. *J Clin Oncol* 2009;**27**(10), 1585.1591.
24. Chang DT, Swaminath A, Kozak M, et al. Stereotactic body radiotherapy for colorectal liver metastases. *Cancer* 2011;**117**(17):4060–9.
25. Hoyer M, Swaminath A, Bydder S, et al. Radiotherapy for liver metastases: a review of evidence. *Int J Radiat Oncol Biol Phys* 2012;**82**(3):1047–57.
26. Withers HR, Taylor JMG, Maciejewski B. The hazard of accelerated tumor clonogen repopulation during radiotherapy. *Acta Oncol* 1988;**27**(2):131–46.
27. Withers HR, Peters LJ, Taylor JMG, et al. Local control of carcinoma of the tonsil by radiation therapy: an analysis of patterns of fractionation in nine institutions. *Int J Radiat Oncol Biol Phys* 1995;**33**(3):549–62.
28. Huang Z, Mayr NA, Gao M, et al. Onset time of tumor repopulation for cervical cancer: first evidence from clinical data. *Int J Radiat Oncol Biol Phys* 2012;**84**(2):478–84.
29. Gao M, Huang Z, Mayr NA, Wang JZ. Tumor repopulation during radiation therapy: stage dependency of onset time for prostate cancer. *Int J Radiat Oncol Biol Phys* 2009;**75**(3):S349.
30. Yom SS. Accelerated repopulation as a cause of radiation treatment failure in non-small cell lung cancer: review of current data and future clinical strategies. *Semin Radiat Oncol* 2015;**25**(2):93–9.
31. Brown JM, Wilson WR. Exploiting tumor hypoxia in cancer treatment. *Nat Rev Cancer* 2004;**4**(6):437–47.
32. Kallman RF. The phenomenon of reoxygenation and its implication for fractionated radiotherapy. *Radiology* 1972;**105**:135.
33. Shibamoto Y, Hashizume C, Baba F, et al. Stereotactic body radiotherapy using a radiobiology-based regimen for stage I nonsmall cell lung cancer. A multicenter study. *Cancer* 2012;**118**(8):2078–84.
34. Videtic GMM, Hu C, Singh AK, et al. A randomized phase 2 study comparing 2 stereotactic body radiation therapy schedules for medically inoperable patients with stage I peripheral non-small cell lung cancer: NRG Oncology RTOG 0915 (NCCTG N0927). *Int J Radiat Oncol Biol Phys* 2015;**93**(4):757–64.
35. Timmerman R, McGarry R, Yiannoutsos, et al. Excessive toxicity when treating central tumors in a phase II study of stereotactic body radiation therapy for medically inoperable early-stage lung cancer. *J Clin Oncol* 2006;**24**(10): 4833–9.
36. Fakiris AJ, McGarry RC, Yiannoutsos CT, et al. Stereotactic body radiation therapy for early-stage non-small-cell lung carcinoma: four-year results of a prospective phase II study. *Int J Radiat Oncol Biol Phys* 2009;**75**(3.):677–82.
37. Timmerman R, Paulus R, Galvin J, et al. Stereotactic body radiation therapy for inoperable early stage lung cancer. *JAMA* 2010;**303**(11):1070–6.
38. Timmerman RD, Hu C, Michalski J, et al. Long-term results of RTOG 0236: a phase II trial of stereotactic body radiation therapy (SBRT) in the treatment of patients with medically inoperable stage I non-small cell lung cancer. *Int J Radiat Oncol Biol Phys* 2014;**90**(1S):S30.
39. Brown MJ, Diehn PM, Loo BW. Stereotactic ablative radiotherapy should be combined with a hypoxic cell radiosensitizer. *Int J Radiat Oncol Biol Phys* 2010;**78**(2):323–7.
40. Choi CW, Cho CK, Yoo SY, et al. Image-guided stereotactic body radiation therapy in patients with isolated para-aortic lymph node metastases from uterine cervical and corpus cancer. *Int J Radiat Oncol Biol Phys* 2009;**74**(1):147–53.
41. Rusthoven KE, Kavanagh BD, Cardenes H, et al. Multi-institutional phase I/II trial of stereotactic body radiation therapy for liver metastases. *J Clin Oncol* 2009;**27**(10):1572–8.
42. Navarria P, De Rose F, Ascolese AM. SBRT for lung oligometastases: who is the perfect candidate? *Rep Pract Oncol Radiother* 2015;**20**(6):446–53.
43. Park H, Lyons JC, Griffin RJ, et al. Apoptosis and cell cycle progression in an acidic environment after irradiation. *Radiat Res* 2000;**153**(3):295–304.
44. Liu Q, Schneider F, Ma L, et al. Sublethal damage (SLD) repair: relation to DNA repair and implications for protracted irradiation with low-energy X-rays. *Int J Radiat Oncol Biol Phys* 2011;**81**(2):S715–6.
45. Shibamoto Y, Miyakawa A, Otsuka S, Iwata H. Radiobiology of hypo-fractionated stereotactic radiotherapy: what are the optimal fractionation schedules? *J Radiat Res* 2016;**57**(S1):i76–82.
46. Fowler JF, Welsh JS, Howard SP. Loss of biological effect in prolonged fraction delivery. *Int J Radiat Oncol Biol Phys* 2004;**59**(1):242–9.
47. Wolbarst AB, Chin LM, Svensson GK. Optimization of radiation therapy: integral-response of a model biological system. *Int J Radiat Oncol Biol Phys* 1982;**8**(10):1761–9.
48. Withers HR, Taylor JMG, Maciejewski B. Treatment volume and tissue tolerance. *Int J Radiat Oncol Biol Phys* 1988;**14**(4):751–9.
49. Pettersson N, Nyman J, Johansson K-A. Radiation-induced rib fractures after hypofractionated stereotactic body radiation therapy of non-small cell lung cancer: a dose- and volume-response analysis. *Radiother Oncol* 2009;**91**(3):360–8.
50. Forquer JA, Fakiris AJ, Timmerman RD, et al. Brachial plexopathy from stereotactic body radiotherapy in early stage NSCLC: dose-limiting toxicity in apical tumor sites. *Radiother Oncol* 2009;**93**(3):408–13.

51. Milano MT, Constine LS, Okunieff P. Normal tissue toxicity after small field hypofractionated stereotactic body radiation. *Radiat Oncol* 2008;**3**:36.
52. Tse RV, Hawkins M, Lockwood G, et al. Phase I study of individualized stereotactic body radiotherapy for hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *J Clin Oncol* 2008;**26**(4):657–64.
53. Borst GR, Ishikawa M, Nijkamp J, et al. Radiation pneumonitis in patients treated for malignant pulmonary lesions with hypofractionated radiation therapy. *Radiother Oncol* 2009;**91**(3):307–13.
54. Grimm J, LaCouture T, Croce R, et al. Dose tolerance limits and dose volume histogram evaluation for stereotactic body radiotherapy. *J Appl Clin Med Phys* 2011;**12**(2):267–92.
55. Rusthoven KE, Kavanach BD, Burri SH, et al. Multi-institutional phase I/II trial of stereotactic body radiation therapy for lung metastases. *J Clin Oncol* 2009;**27**(10):1579–84.
56. Chang EL, Shiu AS, Mendel E, et al. Phase I/II study of stereotactic body radiotherapy for spinal metastasis and its pattern of failure. *J Neurosurg Spine* 2007;**7**(2): 151–60.
57. Russell AH, Clyde C, Wasserman TH, et al. Accelerated hyperfractionated hepatic irradiation in the management of patients with liver metastases: results of the RTOG dose escalating protocol. *Int J Radiat Oncol Biol Phys* 1993;**27**(1):117–23.
58. Eriguchi T, Takeda A, Sanuki N, et al. Acceptable toxicity after stereotactic body radiation therapy for liver tumors adjacent to the central biliary system. *Int J Radiat Oncol Biol Phys* 2013;**85**(4):1006–11.
59. Shaffer JL, Osmundson EC, Visser BC, et al. Stereotactic body radiation therapy and central liver toxicity: a case report. *Pract Radiat Oncol* 2015;**5**(5):282–5.
60. Bentzen SM, Saunders MI, Dische S. Repair halftimes estimated from observations of treatment related morbidity after CHART or conventional radiotherapy in head and neck cancer. *Radiother Oncol* 1999;**53**(3):219–26.
61. King CR, Brooks JD, Gill H, et al. Stereotactic body radiotherapy for localized prostate cancer: interim results of a prospective phase II clinical trial. *Int J Radiat Oncol Biol Phys* 2009;**73**(4):1043–8.
62. Park HJ, Griffin RJ, Hui S, et al. Radiation-induced vascular damage in tumors: implications of vascular damage in ablative hypofractionated radiotherapy (SBRT and SRS). *Radiat Res* 2012;**177**(3):311–27.
63. Garcia-Barros M, Paris F, Cordon-Cardo C, et al. Tumor response to radiotherapy regulated by endothelial cell apoptosis. *Science* 2003;**300**(5622):1155–9.
64. Fuks Z, Kolesnick R. Engaging the vascular component of the tumor response. *Cancer Cell* 2005;**8**(2):89–91.
65. Song CW, Chol LC, Yuan J, et al. Radiobiology of stereotactic body radiation therapy/stereotactic radiosurgery and the linear-quadratic model. *Int J Radiat Oncol Biol Phys* 2013;**87**(1):18–9.
66. Sperduto PW, Song CW, Kirkpatrick JP, Glatstein E. A hypothesis: indirect cell death in the radiosurgery era. *Int J Radiat Oncol Biol Phys* 2015;**91**(1):11–3.
67. Kim M-S, Kim W, Park IH, et al. Radiobiological mechanisms of stereotactic body radiation therapy and stereotactic radiation surgery. *Radiat Oncol J* 2015;**33**(4):265–75.
68. Moding EJ, Castle KD, Perez BA, et al. Tumor cells, but not endothelial cells, mediate eradication of primary sarcomas by stereotactic body radiation therapy. *Sci Transl Med* 2015;**7**:278ra34.
69. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol* 2013;**31**:51–72.
70. Golden EB, Pellicciota I, Demaria S, et al. The convergence of radiation and immunogenic cell death signaling pathways. *Front Oncol* 2012;**2**:88.
71. Lee Y, Auh SL, wang Y, et al. Therapeutic effects of ablative radiation on local tumor require $CD8^+$T cells: changing strategies for cancer treatment. *Blood* 2009;**114**(3):589–95.
72. Formenti SC, Demaria S. Systemic effects of local radiotherapy. *Lancet Oncol* 2009;**10**(7):718–26.
73. Mole R. Whole body irradiation – radiobiology or medicine? *Br J Radiol* 1953;**26**:234–41.
74. Siva S, MacManus MP, Martin RF, Martin OA. Abscopal effects of radiation therapy: a clinical review for the radiobiologist. *Cancer Lett* 2015;**356**(1):82–90.
75. Kaminski JM, Shinohara E, Summers JB, et al. The controversial abscopal effect. *Cancer Treat Rev* 2005;**31**(3):159–72.
76. Demaria S, Ng B, Devitt ML, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biol Phys* 2004;**58**(3):862–70.
77. Hanahan D, Weiberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;**144**(5):646–74.
78. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol* 2004;**22**:329–60.
79. Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. *N Engl J Med* 2012;**366**(10):925–31.
80. Seung SK, Curti BD, Crittenden M, et al. Phase I study of stereotactic body radiotherapy and interleukin-2: tumor and immunological responses. *Sci Transl Med* 2012;**4**, 137ra174.
81. Agassi AM, Myslicki FA, Shulman JM, et al. The promise of combining radiation therapy and immunotherapy: morbidity and toxicity. *Future Oncol* 2014;**10**(15):2319–28.
82. Crittenden M, Kohrt H, Levy R, et al. Current clinical trials testing combinations of immunotherapy and radiation. *Semin Radiat Oncol* 2015;**25**(1):54–64.