Accurate Description of the Cell Survival and Biological Effect at Low and High Doses and \(LET\)'s

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Cell survival modelling/RCR model/Dual Poisson process/Inactivation cross-section/High \(LET\)/Relative biological effectiveness/Mixed beams.

To accurately describe the radiation response over a wide dose and ionization density range Binomial and Poisson statistics have been combined with the recently developed potentially Repairable-Conditionally-Repairable (RCR) damage response model and the combination is shown to have several advantages for the accurate description of the cell survival at both low and very high doses and \(LET\)'s, especially when compared with the classical Linear and Linear Quadratic cell survival models. Interestingly, the potentially and conditionally repairable damage types of the RCR model may also be linked to the two major radiation damage repair pathways of eukaryotic cells namely Non Homologous End Joining (NHEJ) and Homologous Recombination (HR) respectively. In addition it describes the damage interaction of low and high \(LET\) damage in different dose fractions more accurately than any other model (cf. (6) and Fig. 7d). This is of considerable importance when describing the response of tumors and normal tissues during pencil beam scanning with light ion beams where low and high \(LET\) dose fractions from the plateau and Bragg peak can interact synergistically when being delivered quasi simultaneously. In conclusion, considering the unique biological properties of light ion beams such as their increased effect on hypoxic tumors, their microdosimetric energy deposition heterogeneity and their pencil beam energy deposition kernels the largest clinical advantages are obtained with medium \(LET\) beams (\(\approx\) 20–50 eV/nm). This applies even for radiation resistant tumors, at least when the goal is to maximize tumor cure with minimal adverse reactions in normal tissues.

INTRODUCTION

The Linear Quadratic cell survival model is rather accurate in the classical fractionation region from 1.5 to 4 Gy per fraction where the curvature of the survival curve is rather well described by the LQ expression \(S = e^{-\alpha D - \beta D^2}\). However, as the range of applications widens the classical Linear Quadratic cell survival model becomes less and less accurate. This is particularly true at the very high doses employed with single fractions in the dose range from about 5 Gy and above where the traditional \(\beta\)-term saturates and the high dose logarithmic cell survival becomes essentially linear in dose.\(^1-3\) This applies both to low and high \(LET\) beams.\(^4\) Also in the low dose region the LQ model breaks down as seen in Figs. 1 and 2. This is the case in many normal tissues with intact cell cycle regulation, where low dose hypersensitivity is a major concern. This is because side effects in normal tissue at low doses per fraction (< 2 Gy) can be much more severe than expected based on the simple LQ model (cf. (5)). Even if a rather complex modification of the LQ model was introduced recently there is an urgent need for a cell survival model that describe the response very well both at low, medium and high doses. The Repair-Miss-Repair model of Cornelius Tobias (1985) did that to some extent and the Lethal-Potentially-Lethal model of Stanley Curtis (1986) did it even better, but their mathematical formulations are quite complex. More recently several researchers tried to improve the survival model often leading to rather complex equations as those of the first three references above. The Repairable-Conditionally-Repairable survival model\(^5\) has the advantage that the mathematics is quite simple and experimental and clinical data are surprisingly accurately described as seen in

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Furthermore, it has the advantage of being able to account for the two key repair pathways of mammalian cells namely the damage that normally is repaired by the Non Homologous End Joining (NHEJ, mainly for potentially repairable damage) and that requiring the Homologous Recombination processes (HR, mainly for conditionally repairable damage). Fore these reasons, the RCR model has interesting connections to modern molecular cell biology (cf. (7)).
ACCURATE ANALYTICAL DESCRIPTION OF THE BIOLOGICAL EFFECTIVENESS OF LIGHT IONS AT LOW AND HIGH DOSES AND LETS

Cell survival

The cell inactivation is in the first approximation generally exponential since for a given fluence the cell kill is proportional both to the number of cells exposed and the fluence density according to:

\[ -dN = N\sigma_i d\Phi \]  

(1)

where \( \sigma_i \) is the coefficient of proportionality namely the inactivation cross section. This differential equation is directly integrable with the simple solution:

\[ \frac{dN}{N} = \sigma_i \Phi \]

Fig. 3.  

a) The saturation of the high LET cell kill as expressed by the cell inactivation cross section sets in beyond carbon and oxygen ions. At lower ionization densities the ion track is not dense enough to kill the cell after passage through the nucleus (Modified from9)). Since the inactivation cross section is quasi-constant above 200 eV/nm the fluence density of ions determines the survival in that region.  
b) As the cross section in a) saturates the peak biological effectiveness or RBE appears and at higher LET’s the biological effectiveness decreases because of a quasi constant cross section an increasing probability of radical-radical recombination as secondary electrons are generated more and more closely together and the “overkill” sets in. The dashed curves are taken from Eqs. (10) and (13) and describe the average response of multiple experimental data very well. (Modified from 10))

c) The LET dependence of the Oxygen Enhancement Ratio. The analytical Equations (15) and (16) based on the cross section and biological effectiveness in Fig. 3a and b describe the experimental data very well.  
d) Cell survival for high (Bragg peak helium ions) and low LET (X-rays) radiations under well-oxygenated (O2) and hypoxic conditions (N2). The reduced dependence on the oxygenation status of the tumor when using Bragg peak light ions versus sparsely ionizing electrons, photons and protons are clearly seen. The oxygen enhancement ratio is almost unity for Bragg peak helium ions whereas X-rays need to deliver about 2.5 times more dose for the same cell survival and tumor response under hypoxic conditions at the 10% survival level. (Modified from 33)

(33)
so the surviving fraction or survival curve (cf. Figs. 1 and 2) is in the first approximation given by:

\[ S = \frac{N}{N_0} = e^{-\phi D_0} = e^{-D/D_0} = e^{-\alpha D} \]  

where \( \alpha = 1/D_0 \) are the traditional dose proportionality constants of exponential cell survival.

For a given fluence, \( \Phi \), differential in energy \( E \) and restricted mass stopping power \( L_\Delta(E) \) the absorbed dose \( D \) is in the first approximation given by:

\[ D = \int \Phi(E) \frac{L_\Delta(E)}{\rho} dE = \int \Phi(E) L_\Delta(E) dE \frac{\Phi(E)}{\rho} = \frac{L_\Delta}{\rho} \cdot \Phi \]  

where \( \frac{L_\Delta}{\rho} \) by definition is equal to the fluence mean restricted stopping power.

The parameters \( \sigma_i, D_0 \) and \( \alpha \) of equation (3) are thus related according to:

\[ \sigma_i = \frac{L_\Delta}{\rho D_0} = \alpha \frac{L_\Delta}{\rho} \]  

so the inactivation cross section for clonogenic survival can be determined from the logarithmic slope \( 1/D_0 \) of the cell survival curve at high doses. It is basically made up of at least five components according to:

\[ \sigma_i = \sigma_A + \sigma_S + \sigma_M + \sigma_{au} + \sigma_N \]  

namely the cross section for inducing Apoptosis (programmed cell death), Senescence (permanent cell cycle arrest), Mitotic catastrophe (a common cause of death in fractionated radiation therapy of genetically unstable tumor cells), Autophagy (cellular auto digestion) and Necrosis. The cell survival data behind Fig. 3a illustrate how the inactivation cross section, \( \sigma_i \), defined by Eq. (5) saturates approximately at the physical size of the cell nucleus \( (\sigma_n) \) as the ionization density reaches the 150–200 eV/nm region and is so high that a nuclear passage generally leads to cellular inactivation.

To be more accurate this cross section depends on the quasi-circular projected cross section of the cell nucleus \( (\pi r_n^2) \), cf. Fig. 4) and the width of the radial ion beam dose

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**Fig. 4.** Illustration of the relation between the inactivation cross section \( \sigma_i \) and the cross section for a nuclear hit, \( \sigma_0 \), and their dependence on the quasi spherical nuclear size and the approximately cylindrical mean radial dose profile of the ion track. The maximum radial excursion of secondary electrons (cf. Fig. 5) is denoted \( \rho_1 \) whereas the effective inactivation radius is \( \rho \), and is significantly smaller. In Fig. 5 \( \rho_1 \) is at a dose level far below 1 Gy whereas \( \rho \) normally is at a dose level significantly above 10 Gy making \( \sigma_0 \) significantly larger than \( \sigma_i \) and \( a \) larger than \( e \) in Eqs. (8), (9) and (17–20).

**Fig. 5.** Comparison of experimental and calculated radial dose profiles through point monodirectional proton and oxygen beams near the Bragg peak. It is seen that the central axis dose is lower and the radial width narrower for the protons. This is also part of the reason why protons have a lower effective cellular cross section whereas oxygen ions have reached the plateau value where the cross section is practically equal to the molecular size of the DNA in the cell nucleus. Interestingly the high dose region is of a size comparable to the beads on a string nucleosome fiber of 10 nm and the more condensed 30 nm fiber. For protons a comparison is made between the accurate distorted wave calculated profile (solid line: analytical and histogram: Monte Carlo) and experimental data: rhombic.\(^{11}\)
profile \( (r_i) \) at the level of the inactivation dose, generally located in the range above several Gy (cf. Fig. 5), but also on the radial LET distribution of the ion and the mean cord length when crossing the nucleus.

In the first approximation the inactivation cross section in the high LET region can according to Fig. 1 be expected to have a dual Poisson process type LET dependence \( \sigma_i(L) \) of the form:

\[
\sigma_i(L) = \sigma_\infty \left( 1 - e^{-\lambda L} (1 + \lambda L) \right) = \sigma_\infty \left( 1 - \lambda L + \frac{\lambda^2}{2} L^2 - \frac{\lambda^3}{6} L^3 (1 + \lambda L) \right) = \sigma_\infty \left( \frac{(\lambda L)^2}{2} + \cdots \right) = \sigma_\infty \left( 1 - e^{-\lambda L} \right)
\]

where the first expression is based on the assumption that cell kill is described well by a dual or higher order Poisson process mainly induced by dual or higher order double strand breaks but also higher order events such as multiply damaged sites (cf. Fig. 6, (12) and (13)). As seen in Fig. 3 the second part may be a suitable low and high dose approximation previously used in the theory of dual radiation action.\(^{14}\)

It is well known today that 2 Gy of low LET radiation generally induces about 70–80 DNA foci or reparisomes at double strand breaks. However, less than one of them may be lethal since the Survival Fraction at 2 Gy is generally around SF\(_2 \) = 0.5 which is higher than the value with 1 lethal event, on average giving SF\(_2 \) = \( e^{-1} = 0.37 \). In fact SF\(_2 \) = 0.5 corresponds to \(-\ln(0.5) = 0.693 = 0.7 \) lethal events or just under 1% of the 70–80 foci or DSBs. Most of the double strand breaks are thus repaired and in general a close pair of local double strand breaks is at least needed for lethality as shown in Fig. 6.

A close pair of double strand breaks may result in a number of lethal repair problems such as losing a short section of DNA or getting chromosomal cross links. In Eq. (8) it is therefore based on Poisson statistics as shown in Fig. 1 assuming that cells that have none \( (e^{-\lambda L} = e^{-aL\Phi/\rho} = e^{-aD}) \) (cf. Eqs. (17) and (18) below) or just a single double strand break \( (e^{-\lambda L}) \) generally survives and two or more hits are commonly needed for lethality thus including the dual double strand breaks and the more general multiply damaged sites as commonly recognized lethal events. The dual double strand breaks induced by delta electrons are in fact the most common lethal event as shown in more detail in Fig. 6a and by Brahme et al.\(^{12}\). The dual double strand breaks on the periphery of a nucleosome requires full nucleosomal dismantling before the initial repair gene products Ku70 and Ku86 can bind to the free DNA ends since they are almost as large as the nucleosome itself. In this process the precise DNA geometry before the damage was inflicted, may be lost leading to lethality either if it happens in a gene of key importance or if the repair process is not efficient enough to prevent cell death.

![Fig. 6.](image)

*Fig. 6. a) Molecular close up view showing that most of the lethal cell damage of densely ionizing ions is induced by low energy \( \delta \)-electrons in the 200 eV to 1 keV range that can generate severe difficult to repair DNA damage in the cell nucleus such as dual double strand breaks at the periphery of the nucleosome. (cf. (12)) The lower right insert shows that at high doses of low LET the most common DNA segment length corresponds to a single turn of the DNA around the nucleosome as expected from a dual double strand break at the periphery of a nucleosome. Interestingly, the 78 base pair fragments are about twice as common as all other fragment sizes and they should be expected to be even more common with high LET beams having substantially more secondary \( \delta \)-electrons in the keV- to sub keV energy range with very high probability of inducing dual strand breaks. The low RBE of low LET radiation in the late S-phase may be due to a more open chromatix with fewer nucleosomes and thus less dual double strand breaks as well as a more efficient repair using fully developed sister chromatid exchange. b) Illustration of the difference between generally nonlethal single strand and double strand breaks and often lethal dual double strand breaks particularly when the damage is inflicted by low energy \( \delta \)-electrons that mainly produce blunt DNA ends without information where they belonged. The efficient repair of common double strand breaks require dual Poisson survival as shown in Fig. 1.*
importance for cell survival, or if dicentric chromosomes are generated.

According to the above discussion approximately only about 0.9% of the double strand breaks are lethal and thus the inactivation cross section would be more accurately given by:

\[ a) \text{ Total Survival}\]
\[ b) \text{ Lung Epithelial Cell survival}\]
\[ c) \text{ Survival at Varying Combinations of Neon Ions & X-rays}\]
\[ d) \text{ Effective Fractional High LET Dose}\]
\[ e) \text{ RBE (D_{eq})}\]
\[ \sigma_i(L) = \sigma_\infty(1-e^{-\lambda L}(1+0.991\lambda L)) \] (8b)

which is only a small modification of the dual Poisson expression in Fig. 1 and Eq. 8a, but actually adds a small linear term in \( \lambda L \) (0.0091\( \lambda L \)) in Eq. 8a (cf. also Eq. 8c below Eq. 13).

Interestingly we recently estimated the number of sub keV \( \delta \)-electron track ends in the cell nucleus at 2 Gy of low \( \text{LET} \) electrons and photons and found it to be around 1.5. At low \( \text{LET} \) a large portion of the lethal events may thus be caused by such low energy track ends, which we know, can make complex double strand breaks and there are always a constant fraction of them per unit dose.15,16 This is so since the slowing down spectrum at low keV energies is almost the same per unit dose independent of initial electron or photon energy provided the initial energy is above a few hundred keV. This thus explains the constant \( RBE = 1.0 \) for such beams and that the absorbed dose is a good measure of biological effect with low \( \text{LET} \) radiations.

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The constant \( \sigma_\infty \) in Eq. (8a) is the maximum cross section reached at \( \text{LET} \) values around 100–200 eV/nm as seen in Figs. 4 and 5 and it is approximately given by:

\[ \sigma_\infty = \pi(r_s + r)^2 \] (9)

These expressions are further used in connection with the induction of apoptotic cell kill below. The low \( \text{LET} \) approximation of this expression (Eq. (8): \( \sigma_i = \sigma_\infty(\lambda L)^2/2 \) accounts for the reduced cross section and risk for cellular inactivation at low \( \text{LET} \) values since the cell may have many crossing charged particles without a severe energy deposition and cell inactivation (for example for electrons, protons or helium ions).

Eq. (8a) gives us also a very interesting expression for the \( \text{LET} \) dependence of the \( RBE \) at high \( \text{LET} \) values as it is defined as the ratio between the low reference \( (L_0) \) to high \( \text{LET} \) iso-effect doses and it is thus assuming the simple exponential survival according to Eqs. (3), (5) and (8) given by:

Fig. 7. a) Glioma cell survival after increasing doses of \( ^{60}\text{Co} \) and nitrogen ions (N\( ^{7+} \)). The two cell lines M059 J & K of which J is repair deficient (DNA-PK knock out) and respond identically to N\( ^{7+} \) whereas 60Co damage is efficiently repair by the K cells. The responses are well described by the new Potentially Repairable & Conditionally Repairable damage model. This also clearly show that cell lines that differ substantially for low \( \text{LET} \) radiations (60Co) may even have almost identical cell survival for high \( \text{LET} \) beams (N\( ^{7+} \)). The inserts indicate the fractions of the irradiated cells that belong to each subgroup (Venn diagrams). Interestingly, the conditionally repaired cells are rather few at low doses (~0.35 and 0.5 Gy) whereas they dominate the survival at 2 Gy. b) The low dose fast potentially repairable damage may cause a small low dose hypersensitivity plateau on the cell survival curve as seen clearly in b (and less clearly in a), whereas at high doses the conditionally repaired damage dominate the survival curve shape. In normal tissues there is therefore a fractionation window around 2 Gy (\( = 1.5-2.5 \) Gy) per fraction where the least detrimental response is obtained (SF\( _2 = 0.52 \) and D\( _{\text{eff}} = 3.1 \)) for a given dose level in the surrounding tumor volume. The lower dashed curve show how SF\( _2 \) and D\( _{\text{eff}} \) varies with the dose per fraction on the horizontal axis having clear maxima near 2 Gy. This is probably the main reason why in classical radiation therapy where the tumor and normal tissue dose is often rather similar and the dose per fraction should preferably be around 2 Gy. For light ions with largely a low \( \text{LET} \) in normal tissues, such as lithium to carbon ions, the normal tissue dose should also be in this range unless there is a substantial high \( \text{LET} \) dose spill over to critical normal tissue in the tumor region. c) Cell survival at varying combinations of neon ions & X-rays; Solid lines: RCR model Dots: experimental data.\(^{10}\) The formula included in the figure show how the dose weighted mean values of \( a, b \) and \( c \) of the different radiations, i, combine to give the total effect (cf. (6)). d) Variation of the effective high \( \text{LET} \) dose fraction (vertical scale) with the real delivered high \( \text{LET} \) dose fraction (horizontal scale). The convex upper curve illustrate the data in c) (dots) and indicate a clear synergistic action between low and high \( \text{LET} \) when delivered almost simultaneously whereas the lower concave curve\(^{19,20}\) show reduced effectiveness when the high and low \( \text{LET} \) fractions are delivered at significant time intervals, several minutes apart, allowing repair of sub lethal damage between the radiations types. This plot clearly shows that a short treatment interval is desirable with scanned light ion beams to get almost a 30% increase in the synergistic effect between the low and high \( \text{LET} \) dose fraction such as between the peak and plateau part of the dose delivery. This is automatically obtained by fast dynamic range shifters or ridge filters but not necessarily by a single swept pencil beam that is gradually scanning the tumor volume starting from its distal edge and thus may cause a 20–30% reduction in treatment effect due to fast repair processes in the plateau region. A scanning system that irradiates the tumor many times during a few minutes treatment session or preferably a fast longitudinal scan may be the way to relieve this problem. Several studies\(^{18,21,22}\) indicate that the order of high and low \( \text{LET} \) fractions does not matter much. Interestingly, the synergistic effect is highest at high doses per fraction as seen from the uppermost convex curve and data points in Fig. 7d. e) Illustration of the generation of a quasi uniform absorbed dose and cell kill distribution between 21 and 26 cm of depth by combining lithium and carbon ions in suitable ratios to make the cell kill and survival quasi uniform. The small local variations in absorbed dose are due to a somewhat too large longitudinal range modulation (~3 mm) used to clearly illustrate the applied mechanism combining lithium and carbon ion Bragg peaks at each depth. The different panels show the total dose and carbon dose and the lithium dose in the upper row, whereas the cell survival and mean \( \text{LET} \) distribution is shown below. Interestingly, by combining lithium and carbon ions a uniform biological effect, survival and absorbed dose can be obtained for a uniform tumor. The survival was calculated for simultaneous irradiation based on Eqs. (25), (31), (34) and (35) whereas the mean \( \text{LET} \) is more difficult to interpret as it is based both on lithium and carbon ions. f) Illustration on how the \( RBE \) decreases with the applied dose level since the curvature of the low \( \text{LET} \) reference cell survival curve is high at low doses (cf. Figs. 7a–c). When the cell line shows low dose hypersensitivity (cf. Figs. 7b and c) with the reference X-rays the high \( RBE \) disappears at the lowest doses as described rather well by the RCR model.
\begin{equation}
RBE(L) = \frac{D(L)}{D(L)} = \frac{D_0(L)}{D_0} = \frac{\sigma(L)/L}{\sigma_0(L)/L_0} = \frac{1 - e^{-\lambda L}}{(1 + \lambda L)/\lambda L_0}
\end{equation}

and this expression describes the shape of the RBE peak very well as shown in Figs. 3b, 8 and 9. The RBE peak is located at $L_{\text{max}} = 1.79/\lambda$, and the Full Width at Half Maximum, FWHM = 3.5/$\lambda = 2L_{\text{max}}$. The dimensionless functional value at maximum of each member of Eq. 10 is about 0.30 at $\lambda L_0 = 1.79$. Interestingly, $RBE(L)$ in a logarithmic diagram that is as a function of the logarithm of the LET is approximately shaped as a Gaussian function as seen in Figs. 3 and 8. Equation (9) should strictly be used for ions so $L_0$ is the LET of very high-energy ions that are generally generating high-energy $\delta$-electrons with quite low LET values. With low LET photons as reference Eq. (13) below is more accurate.

In the low LET region of high energy photons and electrons the RBE is more accurately derived based on the LET, the mean ionization energy $\overline{W}$, mean cord length, $\overline{t}$, and effective cross section, $\sigma$, of the cell nucleus to get ionized by the particle fluence. The average number of ionizations produced when crossing the cell nucleus is then given by:

\begin{equation}
\begin{aligned}
n &= \frac{\overline{t}}{\overline{W}(L)} \Phi(L) \sigma(L) = \frac{\sigma(L)\overline{t}}{\overline{W}(L)} D(L) = \frac{\overline{t}_0}{\overline{W}_0} \Phi_0 \sigma_0 \\
 &= \frac{D_0}{W_0} \Phi_0 \sigma_0 = \frac{\Phi_0}{W_0} \frac{D_0}{\sigma_0} = \frac{m}{W_0} D_0
\end{aligned}
\end{equation}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{apoptosis.png}
\caption{The inductions of apoptotic cell kill as a function of the LET and the status of the P3 pathway. When some part of the P3 pathway is mutant, the apoptotic fraction is reduced to about half its value for normal wt P3 and non AT cell lines that are intact on the ATM gene upstream of P3. Interestingly the apoptotic fraction peaks at lower LET values due to the higher flux density of ions and apoptotic events per unit-absorbed dose as seen in Figs. 9d and 10a than the RBE.}
\end{figure}

where all but the first equality holds for the reference radiation of LET $L_0$ such as $^{60}\text{Co}$ and the last parts identifies the relation to the absorbed dose and cell nuclear volume or mass of DNA, $m$. For the low LET range in the first approximation the survival is approximately the same at the same number of induced disperse ionizations and double strand breaks in the cell nucleus. The RBE is then given by the ratio of the applied absorbed doses, for iso-effect associated to the fluencies $\Phi(L)$ and $\Phi_0$ according to:

\begin{equation}
RBE(L) = \frac{D_0}{W_0} \Phi_0 \sigma_0 = \frac{\overline{W}_0 \sigma(L)}{W(L) \sigma_0}
\end{equation}

where Eqs. (4) and (11) was applied in the last step in Eq. (12). Since $\overline{W}$ varies rather slowly with the LET the RBE is slowly varying with LET in the low LET region approximately between 0.2–2 eV/nm. The RBE increases quasi linearly in the region of increasing LET beyond a few eV/nm according to Eq. (10) as seen by comparison with Eq. (8). According to Eq. (12) this is not quite true at the lowest LET values (0.2 eV/nm = 2 MeV/gcm–2) where the RBE is almost constant. In order to describe the RBE dependence over the whole LET range from the lowest (0.2 eV/nm, relativistic electrons) to the highest values towards $10^5$ eV/nm and beyond for heavy ions it is therefore necessary to add a small exponential term to account for the low LET electrons producing the low almost constant RBE value independent on the LET due to the high energy low LET $\delta$-electrons set in motion by very high-energy ions. Both these electron types are producing a low quasi-random ionization density rather than the densely ionizing track ends making double DSBs particularly common around the Bragg peak as described by the dual Poisson term:

\begin{equation}
RBE(L) = \frac{\overline{W}_0 \sigma(L)}{W(L) \sigma_0} e^{-k/\lambda L_0} + k(1 - e^{-k/\lambda L_0} (1 + L/L_0)) L_0/L
\end{equation}

where $k$ is given implicitly by Eq. (10), and the characteristic high and low LET values are $L_h = 1/\lambda$ and $L_l = 2L_h \overline{W}_0 \sigma(L)/kW(L) \sigma_0 \leq L_0$ to have a smooth RBE transition to high LET’s somewhat depending on ion species and cell line as seen in Fig. 3b. The function in Eq. (13) thus describes the LET dependence of the RBE very well over a wide LET range by combining the different low and high LET contributions by $\delta$-electrons. The first term is thus due to high-energy low LET $\delta$-rays and the last essentially due to sub keV $\delta$-electrons with dual double strand break induction capability as described in detail in Fig. 6. Based on Eqs. (8b) and (13) it is possible to express the cross section more accurately taking also the high-energy $\delta$-electrons into account according to:

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which makes the expression more valid also for helium, protons and low LET photons and electrons.

Interestingly, based on this expression for the LET dependence it is possible to estimate also the LET dependence of...
the *OER*, or Oxygen Enhancement Ratio, as shown in Figs. 3c and d. If we know the *RBE* at a given survival level for well oxygenated and hypoxic conditions: \( \text{RBE}_{\text{O}} = D_0^\text{O}/D_0^\text{c} \) and \( \text{RBE}_{\text{H}} = D_0^\text{H}/D_0^\text{c} \) and furthermore also the generally quite high low LET *OER* for photons defined by \( \text{OER}^\text{p} = D_\text{H}^\text{p}/D_\text{O}^\text{p} \) for the same cell line (cf. Fig. 3d) it is straightforward to calculate the associated ion *OER* according to:

\[
\text{OER}^\text{I} = D_\text{H}^\text{I}/D_\text{O}^\text{I} = \text{OER}^\text{p}\text{RBE}_{\text{O}}/\text{RBE}_{\text{H}}
\]

(14)

Here the *RBE* ratio can be recognized as the 1/Oxygen Gain Factor where \( \text{OGF} = \text{RBE}_{\text{H}}/\text{RBE}_{\text{O}} \) describing the reduction of dose possible going from photons to light ions. So from the known maximum \( \text{OER}^\text{p} = 3 \) for low LET photons and electrons it is possible through Eq. (14) to calculate the ion \( \text{OER}^\text{I} \) using Eq. (13) for the oxic and hypoxic cells (index O and H respectively) according to:

\[
\text{OER}^\text{I} = \frac{\frac{W_o(L)}{W(L)} e^{L\sigma_o} + k_o(1-e^{-L\sigma_o}(1+0.991L/I_0))I_0/L}{\frac{W_o(L)}{W(L)} e^{L\sigma_o} + k_o(1-e^{-L\sigma_o}(1+0.991L/I_0))I_0/L}
\]

(15)

By power expansion similar to Eq. (8a) and assuming the OER is a simple dose-modifying factor (cf. Fig. 3c) this expression can be approximated very well simply by:

\[
\text{OER}^\text{I} = 1 + (\text{OER}^\text{p} - 1)e^{L(1/\text{RBE})}
\]

(16)

which describes the experimental data\(^{23}\) in Fig. 3c very accurately.

The simple cell survival expressions in Eq. (3) do not really take the effect of the complex cellular repair systems into account. During the 1960s and 1970s this was taken into account using the extrapolation number, \( n \), and defining a quasi threshold dose, \( D_\text{aq} \) at which the extrapolated survival was unity as shown in Fig. 2. To describe the cell survival more accurately at low doses the linear quadratic survival model was later introduced as also shown in that figure. Unfortunately at the very lowest and highest doses there are still deviations as seen by comparison with the experimental data points in Figs. 1 and 2. Interestingly, the effect of the repair system could be taken into account more accurately using the recently developed potentially Repairable–Conditionally Repairable or RCR cell survival model which includes a clear cut repair term (Figs. 1, 2 and 7a, b and\(^{6,7}\)) according to:

\[
S = e^{-\phi} + bDe^{-\phi}
\]

(17)

where the second term includes all cells that are able to correctly repair their damage and the first term only includes those cells that survives due to absence of a hit, so:

\[
e^{-\phi} = e^{-\sigma_0 D}
\]

(18)

and in analogy with Eq. (5) we have:

\[
a = \frac{\sigma_0 D}{\Lambda}
\]

(19)

Here the cross section for a hit, \( \sigma_0 \), is larger than \( \sigma_i \) according to

\[
\sigma_i = \pi(r_h^2 + r_h)^2
\]

(20)

where \( r_h \) is outside the sub lethal ion hit radius that generally is well below the 1 Gy dose level (cf. Fig. 5). Here \( a \) is only weakly dependant on the LET at least in the low LET region since high energy electrons, photons or protons can cross a cell nucleus with a small but finite probability of a hit and the fairly long range of the generated secondary electrons may cause a hit when the primary particle do not even cross the cell nucleus.

Interestingly, in analogy with Eq. (8) the second term in Eq. (17) indicates that many of singly hit damage sites (proportional \( \lambda Le^{-\phi} \) or \( aDDe^{-\phi} \)) are really correctly repaired by the effective cellular repair processes NHEJ and HR and survive the irradiation intact as seen in Figs. 1 and 2. The traditional term “sub lethal damage” thus includes many of the conditionally repairable damage events. In fact, the *RBE* is close to unity as long as the fluence of low energy \( \delta \)-electrons (cf. Fig. 6) per unit dose is quasi constant for these particles (cf. (16), (17)). Since \( \sigma_i \) and \( r_h \) are smaller than \( \sigma_0 \) and \( r_h \), \( a \) must be larger than \( \phi \) as is generally shown by experimental data (cf. Figs. 1, 2 and 4 and (6)).

However, in order to make the survival less than unity \( a \) does not only have to be larger than \( \phi \) but also:

\[
1 \geq e^{-\phi} + bDe^{-\phi} \rightarrow bD \leq e^{-\phi} - e^{((c-a)D)} \rightarrow
\]

\[
0 \leq (a-b)D + \frac{c^3 - (c-a)^3}{6}D^3
\]

(21)

A sufficient condition to fulfill this property is thus \( a \geq b \) and \( \forall n : c^n - (c-a)^n \geq 0 \). This last expression is always fulfilled for odd values of \( n \) since \( a \geq c \) and it is then equal to \( c^n + (a-c)^n \geq 0 \). For even \( n \), \( \left| c^n - (a-c)^n \right| \) which is fulfilled independent of the value of \( n \) if:

\[
a \geq c \geq a/2
\]

(22)

These expressions show that even if Eq. (17) seems to have 3 independent parameters, that is one more than the LQ relation, they obey several conditions (cf. (6)) for details). Interestingly Eq. (17) is very well suited to describe the cell kill both for photons and light ions and various combinations thereof as shown in Figs. 7c and d (cf. (6)). The experimental data for the interaction of X-rays and neon ions of Ng et al.\(^{18}\) agree very well with this new analytical formula (cf. Eqs. (23), (26)). The experimental data in Fig. 7c expressed as the effective high LET dose fraction (see vertical axis in Fig. 7d and\(^{60}\)) are replotted in Fig. 7d as a function of the real high LET dose fraction indicating a strong synergistic effect
between the low and high LET dose fractions specially when delivered simultaneously.

When two radiation modalities are combined on a patient such as low LET photons, electrons and protons and high LET light ions it is essential that the sub lethal damage of all modalities get possibility to interact with each other as shown in Figs. 7c and d. In radiation therapy planning it is very important to be able to calculate this interaction accurately. This is the case not least with light ions where the high energy plateau part of the dose distribution often is of rather low LET whereas the Bragg peak region has a high LET and often is combined with the plateau part specially when using a spread out Bragg peaks (cf. Figs. 7e and 12b). As derived in the initial RCR-publication when there is a LET long time (~24 h) between irradiations the survival level is the product of the individual low and high LET survival levels (cf. the lowest curve in Fig. 7d) according to:

\[ S_{AB} = S_A S_B \]  

(23)

whereas if the irradiations occur simultaneously the interaction is instead given by adding the different \textquotedblleft dose effects\textquotedblright directly according to:

\[ S_{AB} = e^{-\sigma_{ss} D_A} + (b_a D_a + b_b D_b) e^{-\sigma_{ss} D_A} \]  

(24)

This expression really shows how the RCR parameters of different ions and LET's interact. Here the probability of no hit (first term) is similar to Eq. (23) according to the simple exponential hit theory (Eqs. (1)-(3)). However, the last conditional sub lethal repair term is more complex and describes the interaction of damage events that can be significant with high doses of low LET as seen in Figs. 7c and d. Moreover when there is a time interval between the irradiations the sub lethal repair has to be considered. Interestingly, Eq. (24) can be generalized to an arbitrary number of radiation modalities such as the situation during spread out Bragg peak irradiations:

[math]
S_n = e^{-\sigma_{ss} D_{tot}} + \sum_{i=1}^{n} b_i D_i e^{-\sigma_{ss} D_{tot}}
[/math]

where

\[
\sigma_{ss} = \sum_{i=1}^{n} \alpha_i D_i / D_{net}
\]

\[
b_n = \sum_{i=1}^{n} b_i D_i / D_{net}
\]

\[
c_n = \sum_{i=1}^{n} c_i D_i / D_{net}
\]

\[
D_{net} = \sum_{i=1}^{n} D_i
\]

These equations have been used in Figs. 7c and d but also in Fig. 7e to calculate a combined optimal scanning pattern with two different light ions (Li and C) to simultaneously obtain uniform cell survival and absorbed dose.

This is the best way to irradiate extended uniform tumor masses since the regular spread out Bragg peak method results in a rather heterogeneous distribution of the radiation quality with a low dose and very high LET in the distal target volume resulting in microscopic heterogeneity problems as discussed in more detail with Fig. 10.

Based on Eq. (17) the survival with full repair is therefore at low doses well approximated by:

\[ s = e^{-(a-b)D} \left( e^{-\sigma_{ss} D} + b D \right) e^{-\sigma_{ss} D} \]  

\[ = e^{-(a-b)D} \left( 1 + \frac{b}{a} D \right) e^{-\sigma_{ss} D} \]  

(27)

as seen in Figs. 1 and 2, whereas at high doses:

\[ s = \left( e^{-(a-b)D} + b D \right) e^{-\sigma_{ss} D} = b D \left( 1 + \frac{e^{-(a-b)D}}{b D} \right) e^{-\sigma_{ss} D} = b D e^{-\sigma_{ss} D} \]  

(28)

since the exponential term inside the parenthesis rapidly disappears at high doses since \( a \geq c \) and thus

\[ \frac{d \ln s}{d D} = -c + 1 / D \]  

(29)

At very high doses according to Eqs. (3) and (5), we then have:

\[ c = 1 / D_{0,\infty} = \sigma_{ss} \rho / L \]  

(30)

and thus according to Eq. (8)

\[ c(L) = \sigma_{ss} \rho (1 - e^{-3 \lambda (1 + \lambda L)}) / L = c_0 (1 - e^{-L_{\infty}/L} (1 + L_{\infty}/L_n)) L_n / L \]  

(31)

where \( c_0 = \sigma_{ss} \rho \lambda \) and it is assumed that the energy spread of the quasi mono energetic incident ions is so small that \( L = L_{\infty} \) for a given ion species. The shape of this equation is seen in Figs. 3, 8, 9b and c and describes both the LET dependences of the RBE (cf. Eq. (9)) and apoptosis curves quite well (cf. Eq. (36) below). Obviously \( c_0 \) and \( L_n \) will depend on the ion so the response is clearly both ion species and LET dependant. If \( c(L) \) really is proportional to the RBE a more accurate expression is of course obtained by adding the small exponential term as in Eq. (13) above. This may be particularly useful when the LET range of validity is extended to the lowest LET values. A more general term than in Eq. (13) may then be useful to make the fit to \( c(L) \) more accurate according to:

\[ c(L) = c_0 (1 - e^{-L/L_0}) L_n / L + c_1 e^{-L/L_0} \]  

(32)

where \( L_0 = 2 \sigma_{ss} L_n / c_1 \) to make \( c(L) \) quasi constant at low \( L \)-values.

To make \( a \) larger than \( c \) (cf. Eq. (22)) and quasi constant at low LET values (since the hit cross sections Eqs. (19) and (20) and RBE is approximately constant then, as seen in the
discussion between Eqs. (12) and (13)) and slowly decreasing at very high LET values where the inactivation cross section is saturated (Fig. 3a) and radical – radical recombination and overkill takes place, it is again natural to add an exponential term to $c(L)$ which eliminates the linear increase in $c(L)$ at low $L$-values (cf. Eqs. (8), (27) and (28)) and makes $a(L)$ quasi constant at low $L$-values according to:

$$a(L) = \frac{1}{\gamma} \exp \left( -\frac{L}{\gamma} \right)$$

Fig. 10.  a) The increase in the microscopic standard deviation of the mean energy imparted at the local absorbed dose required for tumor cure with decreasing object size and increasing LET or RBE of the ions starting from high energy electrons and photons through protons, helium, lithium, carbon, neutrons and neon ions. With the highest LET beams like neutrons and neon ions the microscopic heterogeneity $\sigma_0$ is so high that microscopic cold spots may leave some tumor clonogens unhit at normally curative doses. b) The reduction in the normalized steepness, $\gamma$, of the dose response relation as the microscopic standard deviation increases. A steeper dose response relation is generally increasing the therapeutic window of radiation therapy since the absorbed dose distribution and the associated therapeutic effect over the therapeutic window can be modulated with greater accuracy with a steep dose response relation for tumor cure.

Fig. 11.  a) Microscopic energy deposition spectra of low and high-energy photons, protons, helium, lithium, carbon and argon ions. It is the LET range around 30 to 50 eV/nm corresponding to the lithium and beryllium peaks that is of highest interest for apoptotic cell kill due to a higher fluence at a given dose level or cell kill. b) Dependence of the variance of the energy deposition ($\sigma^2 = \sigma^y_0 / \gamma^y$) as a function of the frequency mean lineal energy ($\gamma^y$) and different locations on the depth dose curve. Interestingly the Spread Out Bragg Peak (SOBP) is of low variance except near the distal edge where range straggling and very high LET Bragg peak $\gamma^y$-values combine to make $\gamma^y$ high. For the Spread Out Bragg Peak (SOBP) the frequency mean lineal energy or stopping power is fairly constant both for carbon and neon ions but the plateau in front of it and tail has lower $\gamma^y$-values. The unmodulated carbon beam has a much higher clear peak $\gamma^y$ value at its Bragg peak. Neutron, pimeson and proton beams all have a rather high variance in energy deposition. This has the advantage that the region of lowest energy deposition is not so low (few microscopic cold spots) but instead the mean LET is low and so is the biological effectiveness. (cf. (32) updated with recent He, C and Ne data from NIRS)
Eq. (33) is identical to Eq. (31) and the additional term low dose logarithmic slope (a–b small. A further condition that is related to this is that the LET dependence of the apoptotic fraction according to:

\[
a(L) = \sigma_\lambda \rho \lambda \left( \kappa \left( L / L_0 \right)^{\lambda L} + \left( 1 - e^{-\lambda L} \right) \right)
\]

(33)

where \( \kappa \), \( L_0 \) and \( a_0 \) can be selected to fit the low LET value and \( L_0 = 2xL_0 = 2a_0L_0/c_0 \) and \( a_0 = \kappa \sigma_\rho \rho \) so the last part of Eq. (33) is identical to Eq. (31) and the additional term makes \( a(L) \) quasi constant up to LET values towards \( L_0 \) (cf. Fig. 9b).

At low LETs the expression may be simplified further by power expansion similar to Eq. (8) according to:

\[
a(L) = a_0 e^{-(a/L_0)} L^2
\]

(34)

where \( L^2 = 12a_0^2 / 3L_0^2 \) as recently demonstrated for a reduced LET set. To also fulfill Eq. (22) the value of \( \kappa \) and \( a_0 \) should not be too high so the range of validity of \( L \) is not restricted too much (cf. Eq. (22)).

The remaining LET dependant parameter \( b(L) \) describes the amount of repairable damage at increasing LET values. With increasing LET the shape of the cell survival curve gets more and more straight with a decreasing shoulder and curvature. This means that either \( a - c = b/2 \) (cf. Eq. (23), which then results in no second order term in \( D \)) or that \( b \) is very small. A further condition that is related to this is that the low dose logarithmic slope \( (a-b) \) should be similar to that at high doses \( (c) \), which is corresponding to \( a - c = b \). Obviously, if all these conditions are fulfilled simultaneously an almost straight cell survival curve should be expected as generally seen at high LET values.

This is obtained if based on Eqs. (28) and (29) put:

\[
b(L) = 2(a(L) - c(L)) = b_0 e^{-a/L_0}
\]

(35)

This equation really shows how the repairable damage is exponentially decreasing with the LET. Interestingly this also means that the low LET slope \( (a-b) \) and high LET \( (c) \) (cf. Eqs. (17) and (18)) slope are getting more and more close to each other. This is so since \( a - b = c \) implies that \( a - c = b \), which is rapidly approached when \( b \) is getting smaller at high LETs and \( a - c = b/2 \) as seen from Eq. (31) above. Furthermore, this means that there is negligible low dose hyper-sensitivity (requiring \( b/2 \geq a - c \) cf. Eq. (23)) and that there is a strong synergistic interaction between low and high LET damage when delivered simultaneously (cf. Figs. 7c–d and 6).

The equations (17), (30) and (31) have been used in Figs. 9a and b to compare with experimental data for V79 cells irradiated by a wide range carbon ion LETs. A very good fit is seen over the whole LET range of the ion in a, and b show that the LET dependence of the \( a, b \) and \( c \) parameters are in good agreement with the above expressions. Interestingly the \( c \) parameter is in rather close agreement with the LET dependence of the RBE as discussed in more detail recently.24

As seen from the cell survival curves in Fig. 9a the curves get more and more linear as the LET increases. This is according to Fig. 9b due to the fact that fewer and fewer DNA lesions can be faithfully repaired due to increasing severity of the damage and therefore a quasi exponential decrease of the \( b \)-term describing the repairable compartment. This is due to the fact that less and less damage is repairable which is the case with the apoptotic and mitotic catastrophe pathways (cf. Eq. (6) above). This is also the case with senescence, which leads to a permanent cell cycle arrest and no further cell divisions and therefore no clonogenic survival. With increasing LET these types of cell inactivation will dominate more and more and make the cell survival quasi exponential and linear in a logarithmic diagram as clearly seen in Figs. 7c, 9a, 9d and 13a. It is therefore interesting to study the number of cells that are lost by an apoptotic response and due to their reduced inflammatory effects, making apoptosis an ideal pathway for tumor eradication.27

Assuming that the LET dependence of apoptosis is similar to that of inactivation since both are induced by particles with sufficiently high LET producing severe DNA damage (cf. (26) and (27)), the apoptotic cross-section can then be used together with Eq. (8) to formulate an expression for the LET dependence of the apoptotic fraction according to:

\[
\sigma_\lambda (L) = \sigma_\lambda \left( 1 - e^{-\lambda L} \left( 1 + \lambda L \right) \right) = \sigma_\lambda \left( 1 - e^{(\lambda L)^2} / 2 \right)
\]

(36)

where \( \sigma_\lambda (L) \) is the apoptotic part of the inactivation cross section dependent on the LET, \( \sigma_\lambda \) the maximum achievable apoptotic cross section and \( 1/\lambda L = L_0 \) is related to the LET where maximum apoptosis is induced (cf. Eq. (34)). The assumption that two severe events on a single site are needed to trigger the cell to go into apoptosis, corresponds well to the inactivation cross-sections for the dual-Poisson expression as seen in Fig. 8 (cf. (27)).

To model the dose dependence of apoptosis an equation analogous to Eq. (3) can be used. The saturation of apoptotic fraction can then be approximated by:

\[
A_{\rho s} (D) = A_{\rho s} (1 - e^{-D/\rho A}) = A_{\rho s} \left( 1 - e^{-a_\rho \phi} \right)
\]

(37)

where \( A_{\rho s} (D) \) is the induced apoptotic cell fraction at the dose \( D \) and \( A_{\rho s} \) is a LET dependant constant related to the cross section \( \sigma_\lambda \) similar to Eq. (5). An example of an experimental Fluence dependence is shown in Fig. 9c. \( A_{\rho s} \) and \( D_{\rho s} \) were determined though a weighted least square fitting of the data from Meijer et al.25 The value of the constants can be seen in Table 1.
At higher LET, fewer particles contributes to the same dose and therefore the lower LET values have a steeper initial slope as a function of dose, than the higher LET ions. However, when looking at the apoptotic fraction versus fluence all LET’s have the same number of passing particles and there seems to be very small difference in the initial slope between the different LET’s as seen in Fig. 9c. It is also seen that the rapid saturation of the apoptotic fraction is due to a fast reduction of the cell survival with increasing energy deposition at the central absorbed dose. The apoptotic fraction \( f_a \) as a function of the doses and LET in analogy with Eq. (17) is given by:

\[
A_{fr} = A_{\infty} \left(1 - \exp \left[ -\alpha \sigma \rho \left(1 - e^{-\lambda_a L} \left(1 + \lambda_a L \right) \right) / (\lambda_a L) \right] \right)
\]

(38)

Interestingly the equations (8) and (18) that were derived for inactivation of cells also seems to describe their apoptotic response quite well as shown in Fig. 8. However, the apoptotic response does set in at a lower LET due to the fact that a certain energy deposition and LET value need to be reached before apoptosis is more readily induced. Below a certain energy deposition and LET the apoptosis is low above it less and less apoptotic response does set in at a lower LET. For several applications the linear model is sufficiently accurate (cf. (28)).

**FEWER MICROSCOPIC COLD SPOTS AND HIGHER MICROSCOPIC UNIFORMITY OF THE ENERGY DEPOSITION AT INTERMEDIATE LET’S**

The standard deviation of the microscopic energy deposition density as a function of the absorbed dose and object size is presented in Figs. 10 and 11. It is seen that around lithium and beryllium the highest portion of the dose at the Bragg peak is delivered at intermediate energy deposition densities (cf. also Figs. 8, 9, 11, 13 and 14). Figs. 10a and b more importantly demonstrate the effect of increasing microscopic standard deviation (\( \sigma_q \)) on the dose response relation as the LET increases beyond that of the lightest ions. The shallower normalized dose response gradient at high LET’s in Fig. 10b is caused by the microscopic heterogeneity which increase the risk that some tumor cells are missed due to microscopic cold spots even at high normally curative doses. The increased randomness may also cause increased cell kill along the tracks at low doses due to random high dose events (cf. Fig. 10b, (30), (31)). Based on the different dose distributional and biological properties of light ions discussed above it is clear that their optimal usage require some careful considerations as briefly discussed in Fig. 12. For example should the Bragg peak dose delivery mainly be located in the gross tumor so that negligible high LET dose falls on sensitive normal tissues outside the tumor. Furthermore, it is better from a microdosimetric point of view to generate a rather uniform microscopic energy deposition density on the cellular scale as shown in Figs. 10 and 11 and discussed in Fig. 7e.

### Table 1. Fluence and dose response parameters.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Unit</th>
<th>LET 40 eV/nm</th>
<th>LET 80 eV/nm</th>
<th>LET 160 eV/nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A_{\infty} )</td>
<td>%</td>
<td>50</td>
<td>40</td>
<td>19</td>
</tr>
<tr>
<td>( D_0 )</td>
<td>Gy</td>
<td>0.50</td>
<td>0.79</td>
<td>0.43</td>
</tr>
<tr>
<td>( \sigma_a )</td>
<td>( \mu m^2 )</td>
<td>1.3</td>
<td>1.6</td>
<td>3.4</td>
</tr>
<tr>
<td>( A_{\sigma_a} )</td>
<td>%( \mu m^2 )</td>
<td>65</td>
<td>64.0</td>
<td>64.6</td>
</tr>
</tbody>
</table>
LOW DEPENDENCE ON THE PRESENCE OF OXYGEN IN THE TUMOR

The biological effect of low to medium LET radiation is dependent on the local oxygen concentration in the cell since oxygen radicals then mediate a substantial part of the cell kill. The Oxygen Enhancement Ratio (OER) might be defined as the increased radiation resistance in absence of oxygen in the cell. The OER is generally between 2 and 3 for low LET photons, electrons and protons (cf. Fig. 13a). Tumor cells are often living under hypoxic conditions and at least some of these cells are therefore more resistant to conventional low LET radiations. Often 2.5–3 times more low LET dose is therefore needed for eradicating hypoxic tumor cells as compared to well-oxygenated tumor cells. The OER is lower for high LET radiation, which favor the use of light ions with hypoxic tumors (OER = 1.5–1.7). Because the number of double strand breaks is approximately proportional to the absorbed dose, more damage is inflicted to surrounding normal tissues by a curative dose of low LET than by high LET radiation.

In Fig. 3d the reduced dependence on oxygen and more general radiation resistance is clearly shown for Bragg peak helium ions and schematically for high LET ions in general. In Fig. 13 it is shown how the low vascular density and high vascular heterogeneity of a tumor (right panel,34) is produced. It is clearly seen that the hypoxic tumors in the upper left corner are most effectively treated by medium LET carbon ions whereas well oxygenated tumors are best treated (requiring the lowest effective dose) by low LET photons, electrons or light ions. This again demonstrates the advantage of using medium LET ions in the range 20–40 eV/μm at least for hypoxic tumors.

THE CLINICAL ADVANTAGE OF LIGHT IONS: A SIMULTANEOUS LOW OER AND HIGH DOSE, LET AND RBE IN THE TUMOR

From the above discussion of Figs. 3, 4, 5, 7e and 13c and
It is clear that the light ions from lithium to carbon possess very interesting therapeutic properties as they combine in the Bragg peak all the properties one would like in a hypoxic tumor at the same time as they mainly have a low LET character in the surrounding normal tissues. For tumors in organs where an intact internal normal tissue stroma is important for survival the lightest ions with low to slightly elevated LET are most advantageous. An elevated LET may then be
less advantageous for young patients when there is a considerable life expectancy after therapy because there may be a marginally increased risk of a secondary malignancy. The high LET Bragg peak should then be used only in the gross tumor which should be exposed to a high dose level from

**Fig. 13.** a) Illustration how a reduced vascular density and increased randomness in their distribution reduces the oxygenation at a distance from the vessels and increases the number of cells with significant hypoxia ($P_{32} < 5\%$). The color look up table to the right was adopted from that commonly used by hypoxic tissue tracers. b) The vascular model of oxygen diffusion agrees very well with many observed tumors (left half) and normal tissues (right half). c) With increasing hypoxia the $D_{37}$ (and $D_{50}$), the low LET dose causing 37 (and 50) percent tumor cure, increases rapidly. However, the normalized slope of the dose response relation ($\gamma_{37}$) first decreases at low LET values as the hypoxic fraction increases due to the increasing tumor heterogeneity and a dominating small hypoxic compartment and then increases as most of the tumor clonogens become hypoxic. The upper left panel show that the degree of hypoxia in a glioblastoma tumor is much larger than in the normal brain which is contributing to the radiation resistance of these tumors and make light ions the modality of choice at least for the primary tumor (cf. Fig. 13b) The upper right panel show how a small hypoxic compartment can totally dominate a low LET treatment whereas with high LET carbon ions they are no problem and only marginally increase the dose needed for cure. The loss in $\gamma_{37}$ and increase in $D_{37}$ and $D_{50}$ are substantially smaller with high LET lithium or carbon ions as shown in the lower pair of panels. The $D_{37}$ value rises steadily and may be increased 2–3 times whereas the $\gamma_{37}$ first is reduced due to the increased heterogeneity and then reverse and increase as the tumor becomes totally hypoxic. Many clinical tumors are close to the lowest $\gamma$ value ($\approx 2$) and carbon ions brings this low point back up to $\gamma \approx 3.5$ region. d) Overview of how the effective dose response relation is influenced by the LET of the beam from 0.2 eV photons via 25, 50 to 100 eV/nm carbon ions on each tissue hypoxia type taken from a) and b).

**Fig. 14.** Selection of Optimal Radiation Quality Range of the Oxygen Enhancement Ratio (OER), RBE, Apoptotic Fraction (cf. Fig. 8) and the Dose causing 50% tumor cure as a function of the ionization density (LET, upper scale) or densely ionizing dose fraction (lower scale). Lithium to carbon ions are the most interesting and useful light ions for radiation therapy. It is seen that like the RBE the peak apoptotic fraction occurs at increasing LET as the atomic number of the ions increases. Also cell lines with a mutant P53 pathway have a lower level of induced apoptosis. About half the apoptotic fraction is induced by P53 independent pathways.

**Fig. 15.** Comparison of the radiobiological effectiveness (effective LET, RBE and OER) and the lateral and longitudinal dose distributional properties (penumbra) and tumor to superficial tissue dose ratio for uniform parallel opposed beam pelvic irradiation using different radiation beam modalities. The higher the spherical indicator the more effective the beam is for eradication of hypoxic and generally radiation resistant tumors. The approximate costs per typical installation and per patient treated are also indicated. The increase in the Tumor to Normal tissue dose ratio using electron and photon IMRT are indicated. Similar improvements can be obtained using biologically optimized Intensity Modulated or Radiation Quality Modulated light ion Radiation Therapy.
each beam so even if a secondary tumor is induced there is a high probability that it will be sterilized by the same high local dose level that is used to sterilize the primary tumor.

To understand the unique clinical properties of light ions their radiation biological effects are of key importance. With conventional low ionization density or low LET radiations the microscopic dose distribution on the sub-cellular scale is fairly uniform except for the effects of single low energy electron track ends or δ-rays as seen in Fig. 6. With ions the central ion path functions as a source of low energy δ-electrons and at the Bragg peak they are produced at a very high density so multiple δ-electrons contribute to the very high local energy deposition density. In Fig. 5 the mean radial dose profile across a proton and oxygen ion path is shown indicating the larger radial inactivation radius and cross section with oxygen as compared with protons (cf. also Fig. 3).

**CONCLUSIONS**

Both the biological and dose distributional properties of different radiation modalities from low energy photons through high energy electrons and photons to neutrons and light ions are summarized in Figs. 14 and 15 indicating that the intermediate LET light ions are most advantageous in most respects. The quite complex Fig. 14 summarizes the LET (upper horizontal scale) or high LET dose fraction (lower scale) dependence of a number of biological parameters showing that most of the high LET advantages are obtained already at around 30–50 eV/nm or with as little as about one third of the dose in the form of high LET neon ions. With Bragg peak carbon ions this reduction could not be as great as the LET is lower than for neon. With Bragg peak carbon ions probably about half the dose could be low LET.

The 30–50 eV/nm region is close to ideal for the Oxygen Gain Factor (OGF), the OER, RBE and Apoptotic Fraction (ApF) but also with the physical quantities like σμ, the microscopic standard deviation in absorbed dose delivery (cf. Figs. 10a and b). Furthermore the ΔD dose causing 50% probability of tumor cure is still low and the maximum clinically observed steepness of the dose response relation γcmin is still high. The clinically most useful LET-range is thus in this intermediate LET region and not at very high or low LET values. Within the spread out Bragg peak region the LET is reduced, as by necessity some parts of the target volume will receive low LET plateau ion dose. With a rather uniform tumor with regard to cell density and sensitivity it is thus desirable to produce a more uniform LET distribution with a mean LET value around 30–40 eV/nm everywhere. Interestingly, this intermediate LET-region also maximizes the apoptotic cell kill so tumor cells are more effectively eliminated without too much inflammatory response in normal tissues as shown by the dotted (10B) and shaded (12C) curves in Fig. 14. The lower LET at the Bragg peak with boron ions as compared to carbon ions indicate the importance of the fluence density of ions with sufficiently high LET for inducing an apoptotic response (see also Fig. 8) and should be advantageous at least in medium size tumors.

A more classical overview of different particle species is given in Fig. 15 where it is seen that the medium LET ions are also among the most cost efficient ones in clinical use, since the energy needed is lower and the number of treatment fractions are quite low, commonly about 1/2–1/3 of the number with low LET photons and electrons. Optimal particle species are found between helium and carbon for most tumor sites. On the three independent, perpendicular axis in Fig. 15 are plotted: the penumbra width (horizontal-axis), the biological advantage for hypoxic or radiation resistant tumor (vertical axis), and the tumor to normal tissue dose ratio (depth-axis) in spread out Bragg peak beam. The associated costs of a whole installation and for the treatment of a single patient are also indicated. Obviously, treatment modalities with a good tumor to normal tissue dose ratio are most advantageous (deep part of the figure) and so are those with a low penumbra (to the right in the figure) and fairly high biological effectiveness in the tumor region (high up in the figure) so hypoxic or generally radiation resistant tumors are effectively eradicated. Too high a biological effectiveness results in too few ions, a lower apoptotic cell kill and a high microscopic randomness in energy deposition with a resulting shallower dose response relation. It is clear that the group of ions from helium to carbon is most interesting for radiation therapy both from a clinical-dose distributional, biological and economical-cost effectiveness point of view. The low normal tissue RBE at high doses (cf. Fig. 7f), almost straight cell survival curve in the tumor with medium to high LET (cf. Fig. 9a) and the low dose to organs at risk due to the narrow penumbra (cf. Fig. 15), make dose fractionation much more flexible and high doses per fraction very efficient. In general 4–12 fractions are sufficient with light ions and the clinical treatment outcome is often better with a low number of high dose fractions. In addition to the above mentioned reasons a larger vascular tumor effect may also explain this observed clinically advantage not least in organs of a parallel functional normal tissue organization. Organs, such as lung liver and kidney, may therefore tolerate local high doses quite well.

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Cell Survival

for Hypofractionated Irradiation with Therapeutic Carbon Ion Beams.


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