# REPOPULATION OF CANCER CELLS DURING THERAPY: AN IMPORTANT CAUSE OF TREATMENT FAILURE

*John J. Kim* and Ian F. Tannock‡*

Abstract | Radiotherapy and chemotherapy are given in multiple doses, which are spaced out to allow the recovery of normal tissues between treatments. However, surviving cancer cells also proliferate during the intervals between treatments and this process of repopulation is an important cause of treatment failure. Strategies developed to overcome repopulation have improved clinical outcomes, and now new strategies to inhibit repopulation are emerging in parallel with advances in the understanding of underlying biological mechanisms.

FRACTIONATED RADIOTHERAPY
Radiotherapy that is delivered in several discrete dose fractions. Conventional fractionation is usually delivered once daily on weekdays using a dose per fraction of either 1.8 or 2.0 Gray. In accelerated fractionation, treatment is delivered over a shorter total time as a strategy to overcome repopulation.

**Department of Radiation Oncology, Princess Margaret Hospital and University of Toronto, 610 University Avenue, Toronto, ON M5G 2M9, Canada.*
*‡Department of Medical Oncology, Princess Margaret Hospital and University of Toronto, 610 University Avenue, Toronto, ON M5G 2M9, Canada.*
*Correspondence to I.F.T.*
*e-mail:*
*ian.tannock@uhn.on.ca*
doi:10.1038/nrc1650
Published online 20 June 2005

When radiotherapy is used to treat cancer, it is administered in small doses (1.8–2.0 Gray (Gy)), which are given, often daily on weekdays, for 5–7 weeks. The reason for this schedule is to allow the recovery of normal tissues from sub-lethal radiation damage between treatments, and to allow the repopulation of surviving cells in normal tissues during the prolonged overall treatment time. Severe toxic reactions might thereby be avoided. For similar reasons, when cancer is treated using chemotherapy, drugs are often administered with intervals of about 3 weeks between each treatment. This is because many of the drugs cause damage to proliferating haematological precursor cells in the bone marrow, and it takes about three weeks for adequate repopulation from bone-marrow stem cells and their progeny to occur.

Although the overall treatment time allows the repopulation of cells in normal tissues, repopulation of surviving tumour cells also occurs, thereby increasing the number of tumour cells that must be eradicated. There is evidence, reviewed below, that the repopulation of tumour cells limits the effectiveness of radiation therapy, and that tumour-cell repopulation might accelerate during a course of radiotherapy. Tumour-cell repopulation might also limit the effectiveness of chemotherapy, which is not surprising in view of the longer intervals between treatments. However, there are strategies that can selectively inhibit the repopulation of tumour cells during either radiotherapy or chemotherapy, thereby improving the outcome of treatment. Here we review the process of tumour cell repopulation, the mechanisms underlying this process, and approaches that might inhibit it.

## Measuring repopulation during treatment

Tumour-cell repopulation describes the continuing proliferation of surviving tumour stem cells (that is, cells with the capacity to regenerate the tumour) that can occur during a course of FRACTIONATED RADIOTHERAPY or chemotherapy. For fractionated radiotherapy, how well the treatment controls the tumour is determined by several competing factors. Some factors reduce the control of the tumour; for example, tumour cell repopulation and recovery from sublethal radiation injury. Some factors increase the control of the tumour; for instance, reoxygenation of tumour cells and redistribution of cells into more radiosensitive phases of the cell cycle. The number of tumour stem cells present at different times during fractionated radiotherapy will depend on the processes outlined in FIG. 1. Evidence obtained from comparing different radiation fractionation-dose schedules, reviewed below, indicates that repopulation often has a dominant effect on treatment outcome.

Measuring the rate of repopulation of tumour cells during radiotherapy, or between courses of

## Summary

- The repopulation of surviving tumour cells during treatment with radiation and chemotherapy is an important cause of treatment failure.
- The rate of repopulation often increases with time during treatment with either radiotherapy or chemotherapy.
- Mechanisms that underlie tumour repopulation are poorly understood, but might involve the proliferation of tumour cells that are distant from blood vessels and that were destined to die in the absence of cancer treatment.
- Prolongation of a course of fractionated radiotherapy requires a substantial increase in total dose, to counter the effects of accelerated repopulation.
- Accelerated repopulation during successive courses of chemotherapy can lead to an initial response followed by tumour regrowth in the absence of any change in the intrinsic sensitivity of the tumour cells.
- Accelerated radiotherapy and dose-dense chemotherapy (with support from growth factors) represent promising strategies for reducing the effects of repopulation by shortening the overall treatment time.
- The use of molecular-targeted cytostatic agents during radiotherapy, or between courses of chemotherapy, is a promising strategy to inhibit repopulation and thereby to improve therapeutic outcome.


chemotherapy, is challenging because it is difficult to separate tumour stem cells from cells that are morphologically intact but lethally damaged after treatment[1]. Measures of tumour cell proliferation, as assessed by the uptake of markers of DNA synthesis such as bromodeoxyuridine (BrdU), or by using flow cytometry to measure DNA content, will not distinguish viable cells from those destined to die during fractionated radiotherapy. By contrast, the longer intervals between doses of chemotherapy means that assessment of tumour cell proliferation shortly before the next treatment is less likely to be influenced by the presence of lethally damaged cells, and might give useful information about repopulation.

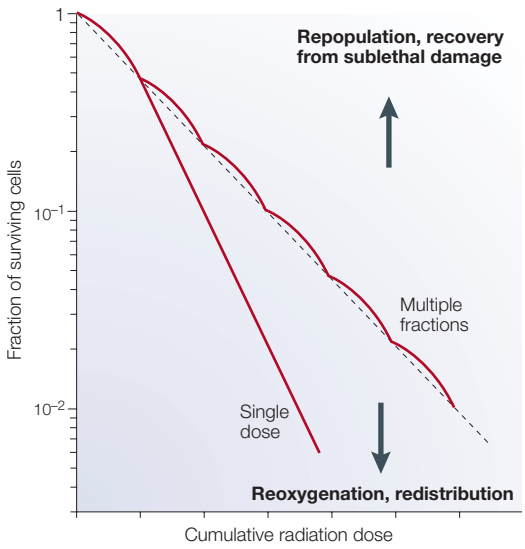


Figure 1 | **Survival curves and processes that influence response to radiotherapy.** Schematic survival cuves after multiple doses or a single dose of radiation are shown. The curves show a reduction in the relative number of surviving cells as the total radiation dose is increased. For single doses, there is an initial shoulder on the curve, which represents the accumulation of sublethal damage, followed by an approximately exponential decrease in survival with linear increments in dose. During the intervals between multiple doses, recovery from sublethal radiation injury, reoxygenation of hypoxic cells (thereby increasing their radiosensitivity), redistribution of cells into more radiosensitive phases of the cell cycle and repopulation all have an effect on cell survival. These processes take place throughout the course of fractionated radiation, but not necessarily at a constant rate. Evidence reviewed in this article indicates that repopulation is an important process that influences outcome after treatment.

For transplantable human or rodent tumours, the number of colony-forming (CLONOGENIC) cells can be estimated by excising tumours at various times during treatment, making a single-cell suspension, and assessing colony formation *in vitro*, under the assumption that this identifies tumour stem cells *in vivo*. For experimental tumours, an alternative estimate of the number of stem cells can be obtained from the average single radiation dose needed to cure the tumour. In this assay, several tumours in mice that have had identical treatments with either fractionated radiotherapy or with chemotherapy subsequently receive a range of single radiation doses. Normally, tumours contain an unknown mixture of aerobic and HYPOXIC CELLS (with the hypoxic cells being approximately three-fold less sensitive to radiation), so the single radiation dose is given under hypoxic conditions (for example, by applying a clamp to prevent blood flow to the tumour) when all cells will have the same intrinsic radiation sensitivity. The dose required to control half of the tumours (that is, the 50% TUMOUR CONTROL DOSE, or $TCD_{50}$) is then measured. Higher values of $TCD_{50}$ relate directly to larger numbers of stem cells in the tumour.

Because of repopulation, the total radiation dose in a fractionated treatment must be increased to control tumours as total treatment time is prolonged[2] (FIG. 2). So, clinical data relating tumour control to treatment duration can provide indirect estimates of the rate of repopulation of surviving tumour stem cells. Repopulation can then be characterized by the increase in radiation dose needed to maintain tumour control for every day that radiotherapy is prolonged. The doubling time of surviving tumour stem cells can also be estimated from the relationship between the total dose required to maintain tumour control and the duration of treatment.

CLONOGENIC CELL
A tumour cell that has the ability to proliferate and produce a substantial number of progeny. Clonogenic cells are usually assayed by allowing them to form colonies. Clonogenic cells are likely to represent tumour stem cells that have the ability to regenerate the tumour and lead to death of the host.

HYPOXIC CELLS
The imperfect vasculature in solid tumours leads to the presence of tumour cells that exist in a microenvironment where the oxygen concentration is very low. Such hypoxic tumour cells are 2–3 fold less sensitive to radiation than well-oxygenated cells.

50% TUMOUR CONTROL DOSE
The dose of radiation that will lead to local control of 50% of tumours. When delivered under hypoxic conditions (to eliminate variable radiosensitivity of tumour cells due to varying levels of oxygen), a change in the $TCD_{50}$ can be used to estimate the change in the number of clonogenic tumour cells that are present.

### Repopulation during radiotherapy

***Rodent tumour models.*** Malaise and Tubiana[3] first demonstrated that regrowth of a transplantable mouse fibrosarcoma was faster after a single dose of radiation than growth of non-irradiated control tumours, and others have reported similar findings[4,5]. In many solid tumours, the rate of growth decreases with time, and can be approximated by the GOMPERTZ EQUATION. In a transplantable rat tumour, after an initial lag period, repopulation of clonogenic tumour cells is faster than controls and increases with the dose of radiation given[5]. However, control and regrowth curves can be fitted by the same Gompertzian curve when adjusted for an initial lag period and for the estimated number of surviving clonogenic cells immediately after irradiation.

When fractionated radiation has been delivered to rodent tumours, using a clinically relevant dose per fraction of about 2 Gy, results have been inconsistent. For the transplantable rat tumour, irradiated either at the implanted site or as lung metastases after intravenous injection with tumour cells, the number of clonogenic tumour cells increases but the rate of increase is slower than in control tumours[6,7]. Reoxygenation of clonogenic cells during radiotherapy can increase their radiosensitivity[8]**.** When radiation has been delivered under hypoxic conditions, accelerated repopulation has been demonstrated in several tumours during fractionated radiotherapy[9–12]. Other investigators have reported similar repopulation kinetics under aerobic and hypoxic conditions[13,14].

Repopulation has also been studied during fractionated radiotherapy delivered to human tumour xenografts in immune-deficient mice. For a poorly differentiated squamous cell carcinoma xenograft, repopulation kinetics could best be fitted to a model in which there was initially minimal repopulation, followed by accelerated repopulation after about 3 weeks of treatment[13]. Conversely, a change in repopulation kinetics over time was not observed in a moderately well-differentiated squamous cell carcinoma[15]. When a human soft-tissue sarcoma xenograft was irradiated with different fractionation schedules, repopulation was substantially greater for the most protracted schedule, and was faster than growth in untreated tumours[16]. Increasing overall treatment time by the introduction of a treatment break resulted in decreased control of human cervical carcinoma xenografts[17]. For xenografts derived from human melanoma, squamous cell carcinoma and brain tumours, the radiation dose per day required to overcome repopulation of tumour cells was in the range of 0.5–0.9 Gy per day[18]. This dose range is similar to that derived from analysis of different schedules of clinical radiotherapy, discussed below.

Despite some variability, there is strong evidence for accelerated repopulation during fractionated radiotherapy given to tumours in animals. Representative results are summarised in TABLE 1.


Figure 2 | **The relationship between total dose of radiation to control 50% of oropharyngeal cancers and duration of fractionated radiotherapy.** For courses of radiotherapy that last up to about one month the line is flat (lag phase), indicating that there is little or no repopulation in the tumour. When treatment time is extended beyond about 1 month, repopulation between dose fractions increases the number of tumour cells that must be killed, which means that increasing doses of radiation must be given. Line is fitted to data for oropharyngeal cancers. Modified, with permission, from REF. 2 © (1988) Taylor and Francis.

GOMPERTZ EQUATION
An equation that has been used to fit tumour growth curves. The equation is $V = V_0\exp(a(1-\exp(-bt)))$ where V is tumour volume, $t$ is time and a, b and $V_0$ are constants. The equation describes a decreasing rate of tumour growth with increasing time, as is commonly observed.

***Clinical radiotherapy.*** In an important paper, Withers and colleagues[2] analysed pooled clinical data, from different radiotherapy institutions, for total radiation doses required to control 50% (that is, $TCD_{50}$) of squamous cell carcinomas of the head and neck. The marked increase in $TCD_{50}$ if the treatment lasted more than 4 weeks was ascribed to accelerated repopulation (FIG. 2). Although these tumours are usually slow-growing with typical doubling times of 2–3 months, the estimated doubling time of surviving clonogenic cells decreased to about 4 days. Bentzen and Thames[19] indicated that these results should be interpreted with caution because not all institutions used a standard dose of 2 Gy per fraction, and several assumptions were made in estimating $TCD_{50}$. However, an evaluation of results for tonsillar carcinoma from nine institutions showed similar results[20].

For human tumours, estimates of the doubling time of surviving tumour cells during radiotherapy are in the range of 4–8 days[2,21,22]. For epithelial cancers the probability that tumours will grow out of control increases by about 1.0–1.5% for each day that treatment is prolonged beyond a certain time period, which is typically in the range of 4–6 weeks, but might be tumour specific[20,23,24]. The added radiation dose required to overcome repopulation is in the range of 0.5–1.0 Gy per day of treatment prolongation[2,20,22,23,25]. These data are derived from retrospective reviews of radiation treatment protocols, and might be confounded by the factors outlined in FIG 1. However, Withers and Peters[26] analysed the results of a large prospective randomized trial that compared accelerated fractionated radiotherapy (delivered over 6 weeks) with conventional fractionated radiotherapy (delivered over 7 weeks) for head and neck cancer, and found similar parameters of repopulation[27].

Table 1 | **Repopulation during fractionated radiotherapy for experimental tumours**

| Tumour | Evidence for accelerated repopulation | References |
|---|---|---|
| ***Mouse*** | | |
| C3H mammary carcinoma | Radiation dose to control 50% of tumours increased when total treatment time exceeded 9 days. | 12 |
| MCA-4 mammary carcinoma | Estimation of doubling time of clonogenic cells was faster in irradiated than in un-irradiated tumours | 9 |
| SSK fibrosarcoma; AT7 adenocarcinoma; AT478 SC carcinoma | Estimation of doubling time of clonogenic cells during treatment was faster than pre-treatment volume doubling time or potential doubling time. Cell proliferation assessed by IdU uptake in tumours did not correlate with radiobiological data | 10 |
| SCCVII SC carcinoma; B16-F1 melanoma; RIF-1, KHT-C and KHT-LP1 fibrosarcomas | For all cell lines, surviving fraction of clonogenic cells was greater for a radiation schedule that allowed repopulation than one that did not. SCCVII and B16-F1 tumours showed accelerated repopulation | 11 |
| ***Human*** | | |
| Melanoma xenograft cell lines: EF and VN | Number of clonogenic cells as a function of total dose. 2.0–2.2 Gy per day required to overcome repopulation. Doubling time of clonogenic cells during radiation was faster than pre-treatment potential doubling time | 18 |
| HSTS26T soft tissue sarcoma | Radiation dose to control 50% of tumours increased with overall treatment time. 1.35 Gy per day was required to overcome repopulation | 16 |
| SiHi cervical SC carcinoma | Rapid repopulation after radiation due to more clonogenic cells | 17 |
| FaDu SC carcinoma | Radiation dose to control 50% of tumours as a function of fraction number and overall treatment time was consistent with a biphasic model of repopulation. Repopulation accelerated as time increased | 13 |

SC, squamous cell; IdU, iododeoxyuridine.

The detrimental effects of extending overall treatment time for tumour control is established for many malignancies, including squamous cell carcinomas of the larynx[23] and pharynx[25], carcinoma of the cervix[24], and bladder cancer[22]. This effect is observed both for primary radiation treatment and for postoperative radiotherapy[28].

## Repopulation during chemotherapy

There have been few studies of repopulation in rodent tumours after chemotherapy (TABLE 2). Five studies of animal tumours, each treated with single-agent chemotherapy[29–33], and two studies of multicellular TUMOUR SPHEROIDS[34,35], all consistently demonstrate increased repopulation after chemotherapy.

Even less is known about repopulation in human tumours after chemotherapy. Studies have been limited to evaluating cellular proliferation from biopsy samples taken at various intervals after the last course of chemotherapy. In a study of patients with oropharyngeal cancer, Bourhis and colleagues[36] estimated the potential doubling time of viable cells in tumour biopsy samples after chemotherapy. They found evidence for accelerated repopulation as compared with cell proliferation in untreated tumours, and concluded that accelerated repopulation was associated with a poor response to treatment. In contrast, in a pilot study of patients treated with platinum-based chemotherapy for ovarian cancer, Davis and colleagues[37] found that at variable times after the last chemotherapy (mean 33 days) the PROLIFERATIVE INDEX (assessed by staining for a nuclear antigen associated with proliferation, Ki67) was increased in four patients, reduced in 12 and unchanged in five patients.

TUMOUR SPHEROIDS
Spherical aggregates of tumour cells that can be grown in tissue culture. Spheroids retain many properties of solid tumours, including tight junctions between epithelial cells, the generation of an extracellular matrix, and gradients of nutrient concentration and proliferative rate from the outer to inner layers.

PROLIFERATIVE INDEX
The proportion of cells in a population that are identified by a marker of cell proliferation such as Ki67, or by the uptake of bromodeoxyuridine (BrdU).

## Models of repopulation

*Radiotherapy*. Solid tumours have a high spontaneous rate of cell death, which in human cancers is often 80–90% of the rate of cell production[38]. The potential doubling time of a tumour (that is, the time it would take to double in volume in the absence of cell loss or death) is therefore much shorter than its actual doubling time. Older studies established that the rate of cell proliferation in untreated tumours decreases with increasing distance from tumour blood vessels, with a high rate of death in the quiescent population, related in part to deprivation of oxygen and other nutrients[39–41]. In a model proposed by Fowler[42], well-oxygenated cells proximal to the blood vessels die and are removed following radiotherapy. Consequently, the nutritional and oxygen status of the remaining cells improves, with the result that the rate of spontaneous cell death decreases. This is magnified over a course of fractionated radiotherapy. The decrease in the spontaneous death of tumour cells is then the predominant factor, which leads to accelerated repopulation (FIG. 3a).

Kummermerhr and Trott[43] have proposed a model based on proliferation and differentiation during the renewal of normal tissues. In this model, tumour stem cells normally produce more of both themselves and cells that undergo terminal differentiation. When repopulation occurs during radiotherapy, a greater proportion of the progeny of stem cells is assumed to retain clonogenic capacity (FIG. 3b). The rate of proliferation of clonogenic tumour cells might also be faster, with fewer aborted cell divisions[44]. This model is derived from data from normal murine squamous epithelium, which also shows altered repopulation kinetics after ionising radiation[45,46]. Alterations in the

Table 2 | **Evidence for accelerated repopulation in tumours following treatment with chemotherapy**

| Tumour | Evidence for accelerated repopulation | References |
|---|---|---|
| B16 melanoma (mouse) | Quantification of clonogenic cells as a function of time after chemotherapy. Greater response to cyclophosphamide correlates with delayed repopulation as compared with CCNU | 29 |
| 9L gliosarcoma (rat) | Quantification of clonogenic cells as a function of time after treatment with BCNU. Dose-dependent delays in complete repopulation correlate with increases in animal life-span | 30,31 |
| SA-NH sarcoma (mouse) | Estimation of number of clonogenic cells as a function of time after delivery of cyclophosphamide by measuring the radiation dose required to control 50% of tumours ($TCD_{50}$) under hypoxic conditions. After a delay, doubling time decreases to half that of untreated tumours | 32 |
| EMT-6 and MXT mammary carcinomas (mouse) | Assessment of tumour cells incorporating BrdU at 1 week intervals after 1, 2 and 3 courses of chemotherapy with cyclophosphamide or 5-FU. BrdU labeling index higher than in control tumours | 33 |
| V79* | Initial shrinkage followed by re-growth during daily treatments with cisplatin. Re-growth due to repopulation from originally quiescent cells | 34,35 |
| Oropharyngeal cancer (human) | Proportion of S-phase cells increased, and potential doubling time decreased, after induction chemotherapy | 36 |

*Chinese hamster fibroblast cells cultured as spheroids. CCNU, lomustine; BCNU, carmustine; BrdU, bromodeoxyuridine; 5-FU, 5-fluorouracil.

cellular microenvironment might also have a role in influencing such mechanisms and might be relevant to both models illustrated in FIG. 3 (REFS. 13,47).

The molecular mechanisms that underlie accelerated repopulation during radiotherapy are not well understood. Ionising radiation has been shown to activate the epidermal growth factor receptor (EGFR) and other members of the ERBB family of tyrosine kinases, leading to activation of mitogen activated protein kinase (MAPK) pathways and the stimulation of cellular proliferation[48–52]. Schmidt-Ullrich and colleagues[53] demonstrated that ionising radiation induces the proliferation of human squamous cell carcinoma; this response was mediated through EGFR autophosphorylation and was observed over a clinically relevant dose range. The levels of EGFR and cyclin D1, a downstream effector of EGFR, were found to correlate with radiocurability of nine murine epithelial carcinoma cell lines[54,55]. High EGFR levels have also been correlated with poorer clinical outcomes following radiotherapy[56,57] and the prognostic significance of EGFR expression might depend on overall treatment time[58], consistent with a role for EGFR and downstream signalling pathways in accelerated repopulation[49]. Targeting the EGFR, or pathways stimulated by it, might be a logical way of inhibiting tumour-cell repopulation during radiotherapy (see below).

***Chemotherapy.*** Norton and Simon proposed a mathematical model to describe the repopulation that occurs after treatment with chemotherapy[59,60]; they assumed that tumour growth would follow a Gompertzian curve, so that the rate of regrowth would be faster after shrinkage induced by treatment. However, changes in the volume of tumours might occur slowly even after effective chemotherapy, and changes in the number of and the proliferative rate of the surviving tumour stem cells are important in determining the importance of repopulation.

In FIG. 4, we have modelled repopulation during courses of chemotherapy given at three-week intervals, using the assumptions that: (a) the rate of repopulation remains constant (FIG. 4a); (b) the rate of repopulation increases in the intervals between successive courses of treatment (accelerating repopulation) (FIG. 4b); and (c) chemotherapy inhibits proliferation at short intervals after treatment but subsequent repopulation accelerates between successive cycles of chemotherapy[61] (FIG. 4c). It is assumed in FIG. 4 that the intrinsic drug sensitivity of the tumour cells does not change. In practice, increased cellular sensitivity to cytotoxic drugs owing to more rapid proliferation might be countered by the tendency to select cells that are intrinsically more resistant.

Even if the rate of proliferation of surviving tumour cells remains similar to that before treatment, repopulation will affect the effectiveness of chemotherapy. Many common solid tumours show initial response followed by regrowth after further chemotherapy. Models that incorporate accelerated repopulation can describe this acquired drug resistance without the need to assume any change in intrinsic cellular chemosensitivity. The models shown in FIG. 4 predict that repopulation will have a substantial effect on the overall level of cell kill. This effect is particularly relevant when adjuvant chemotherapy is given post-operatively to eradicate micro-metastases: the net cell kill from adjuvant chemotherapy for breast cancer, for example, might reduce cell survival only to the order of 1% (REF. 62).

Durand and colleagues[34,35] investigated the spatial origin of cells that participate in accelerated repopulation after treatment of multicellular tumour spheroids by chemotherapy. There is a gradient of decreasing cell proliferation with increasing distance from the surface of spheroids, similar to that from tumour blood vessels[63,64]. Most anticancer drugs are preferentially toxic to proliferating cells, and many drugs have poor penetration into solid tissue[65–69]. Cells near to the periphery of spheroids were therefore more likely to be killed by chemotherapy, and subsequent repopulation occurred because of entry into the cell cycle of originally quiescent cells near the centre of spheroids[34,35], probably owing to improved nutrition. A recent study evaluated the proliferation of cells in a human colon cancer xenograft by measuring the

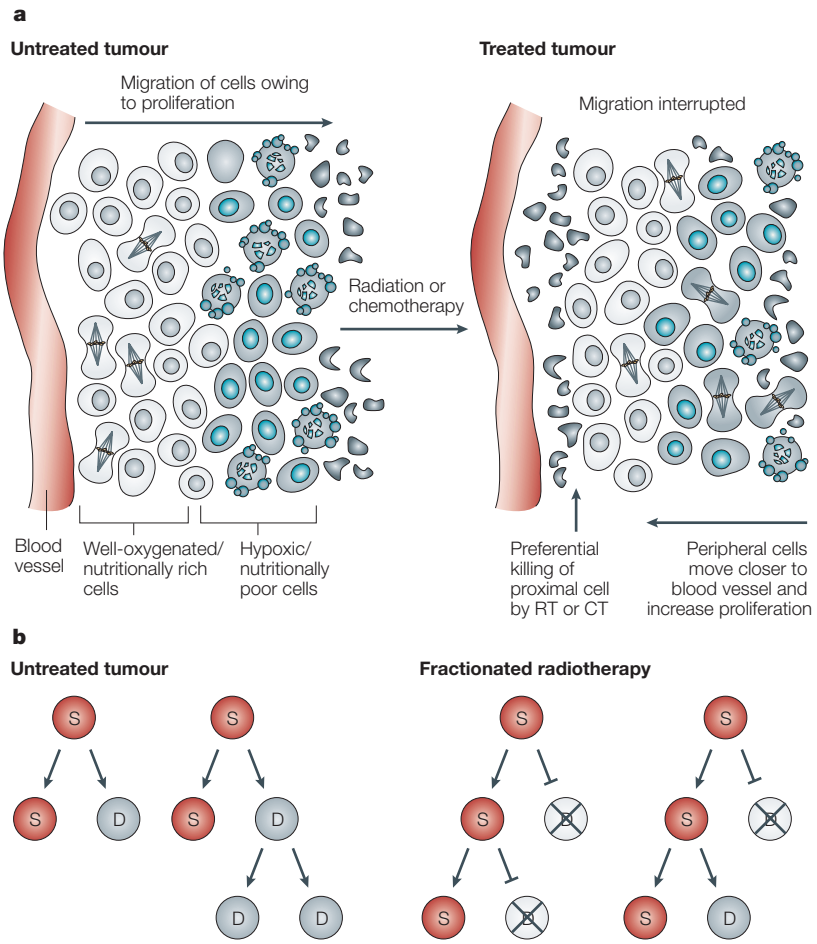


Figure 3 | **Models for repopulation. a** | In an untreated tumour, cells that have migrated further away from blood vessels, as a result of proliferation, will become depleted of oxygen and other nutrients. These peripheral cells will tend to die spontaneously. Radiotherapy (because of selective effects against well-oxygenated cells) and chemotherapy (because of higher drug concentration and selective effects against proliferating cells) are more toxic to cells that are close to blood vessels in tumours. As a result, the migration of cells away from vessels and the rate of spontaneous cell death are reduced. Repopulation occurs from the more distal cells, which tend to be spared by treatment. RT, radiotherapy; CT, chemotherapy. **b** | After fractionated radiotherapy, the relative production of tumour stem (S) cells is increased compared with the production of terminally differentiated (D) cells.

uptake of BrdU after treatment with gemcitabine. Tumour blood vessels were stained with an antibody to the endothelial marker, CD31, and regions of hypoxia were identified by an antibody to pimonidazole, an agent that is selectively taken up by hypoxic cells[70]. Initially, gemcitabine inhibited the proliferation of most tumour cells, but repopulation was observed starting from cells that were more distant from tumour blood vessels, and which had lower rates of proliferation in the untreated tumour.

The above studies are consistent with the model shown in FIG. 3a, which might apply to repopulation after either radiotherapy or chemotherapy. Following chemotherapy, tumour cells close to blood vessels are most likely to be killed because of their higher rate of proliferation (and resultant chemosensitivity) and better drug access; when these cells die or stop metabolising, nutrition of the more distant cells improves, death of the distal cells decreases, and the distal cells re-enter the cell cycle and repopulate the tumour. This model provides a mechanism for the paradox that some tumour cells might survive that would have died in the absence of treatment.

The proliferation of tumour cells after chemotherapy depends ultimately on the activation of cyclin/cyclin-dependent kinase complexes that control the entry of cells into the cell cycle and their passage through the cell cycle. Like after radiotherapy, activation of these proteins might occur through signalling from receptors, such as the EGFR, but little is known about changes in activity of these pathways in tumours treated with chemotherapy.

## Strategies to inhibit repopulation

Strategies that might inhibit the repopulation of tumour cells during either radiotherapy or chemotherapy have the potential to improve the outcome of cancer treatment. Such strategies must be relatively specific for tumour cells, compared with their effects on dose-limiting normal tissues, to improve therapeutic outcome. Promising strategies include those that modify the dose-schedule of treatment (accelerated radiotherapy and dose-dense chemotherapy) and those that use CYTOSTATIC AGENTS to inhibit repopulation.

*Altered radiotherapy fractionation*. Accelerated fractionation reduces the overall treatment time, thereby providing less opportunity for the repopulation of tumour cells[71,72]; using this strategy, the fractional doses are given more than once daily, and/or treatment is continued during weekends. In general, these schedules also increase acute normal tissue toxicity, because there is less time for the repopulation of normal tissue.

Accelerated fractionation has improved local control of Burkitt's lymphoma, a tumour that is known to proliferate rapidly[73]. Accelerated fractionation has been used most extensively for treatment of squamous cell carcinomas of the head and neck, for which a meta-analysis reported a small survival benefit and increased loco-regional control[74]. Two Danish studies, DAHANCA 6 and 7, compared the outcomes of using six daily fractions per week with the conventional five fractions per week, thereby shortening overall treatment time by 7 days. All patients received nimorazole, an agent that sensitizes hypoxic cells to radiation. There was improvement in local control and disease-specific survival[75]. The Radiation Therapy Oncology Group (RTOG) compared a conventional fractionation schedule with two schedules of accelerated fractionation in a large randomized trial; only the accelerated schedule without a planned treatment break showed improved loco-regional tumour control[27]. Another randomized trial showed similar results[76], but two others demonstrated no benefit, perhaps because the total radiation dose was reduced[77,78]. When accelerated fractionation is used to minimise the effects of repopulation in tumours, split-course radiation (that is, with a break of one or more weeks in the middle of a course of treatment) should not be used, because it will allow

CYTOSTATIC AGENT
An agent for which the principle effect is to stop cells from proliferating, rather than directly causing their death.

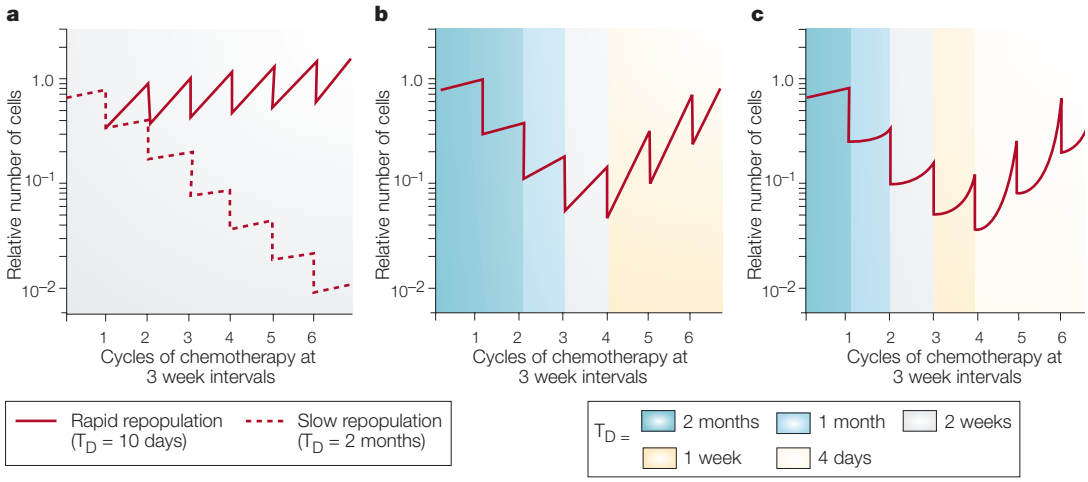


Figure 4 | **Model curves that illustrate the potential effects of repopulation on the total number of cells present in a tumour at different times during chemotherapy, relative to the start of treatment.** It is assumed that 70% of tumour cells are killed after each administration of chemotherapy, which is given at 3-week intervals. **a** | Assumes a constant rate or repopulation of surviving tumour cells between treatments, characterised by a doubling time of either 10 days or 2 months. **b** | Assumes accelerating repopulation of surviving tumour cells between successive courses of chemotherapy, characterised by the indicated doubling times. **c** | Assumes a delay in onset of repopulation after each cycle of chemotherapy, followed by accelerating repopulation of surviving tumour cells with the indicated doubling times. Note that accelerated repopulation can lead to the remission and regrowth of tumours during chemotherapy, as is commonly observed in clinical practice, without any change in the intrinsic chemo-sensitivity of the tumour cells. $T_D$, cell doubling time. Modified, with permission, from REF. 61 © (2000) Lancet Publishing Group.

repopulation to occur, and total radiation doses should be near or at conventional levels.

By contrast to accelerated fractionation, hyperfractionation delivers multiple smaller fractions per day, using conventional overall treatment times, to allow greater normal tissue recovery to occur in each interval between treatments. Accelerated fractionation and hyperfractionation are often combined. In two large UK trials, continuous hyperfractionated accelerated radiotherapy (CHART, in which three daily doses are given continuously without weekend breaks over 12 days) was compared with standard fractionation. The total radiation dose was reduced in the patients given CHART to prevent severe normal tissue toxicity. Improved local control and overall survival was found for non-small cell lung cancer, but not for head and neck cancer[78–80]. However, the CHART experience of head and neck cancer does provide indirect evidence for repopulation using standard radiation dose-schedules — accelerated fractionation with a reduced total dose resulted in similar tumour control to that obtained with standard fractionation.

***Dose-dense chemotherapy.*** The interval between courses of chemotherapy is determined by the requirement that the bone marrow repopulate the white cells and platelets in the blood before the next treatment cycle, thereby minimising the chance of infection or bleeding. The availability of growth factors such as granulocyte-colony stimulating factor (G-CSF, also known as filgrastrim) allows accelerated repopulation of the bone marrow, such that courses of chemotherapy can be given safely at 2-week instead of 3-week intervals. Randomized clinical trials for which 2-week schedules with growth factors were compared with standard 3- or 4-week schedules have shown improved survival when used as adjuvant treatment for breast cancer[81] and for treatment of non-Hodgkin's lymphoma[82]. There were similar trends for treatment of advanced bladder cancer[83], although not in a smaller trial for locally advanced breast cancer[84]. These trials did not report increases in normal tissue toxicity compared with conventional schedules. These encouraging results need confirmation before their widespread adoption, especially because the modified treatment is expensive.

***Combined radiotherapy and chemotherapy.*** There have been several trials in which chemotherapy has been given concurrently with radiation therapy, and individual randomized trials and meta-analyses have demonstrated improvement in survival following primary treatment of cancers of the head and neck and uterine cervix[85–87]. Promising results have also been obtained for stage III lung cancer, oesophageal cancer, bladder cancer and others. In general, strategies in which chemotherapy is given before radiotherapy have not been shown to be therapeutically beneficial, perhaps because initial chemotherapy stimulates repopulation throughout the subsequent radiotherapy.

The mechanisms that underlie the benefits of concurrent chemotherapy and radiotherapy are complex: they might include additive anti-tumour effects and/or radio-sensitization, but it is unclear how this would be selective for the tumour rather than for normal tissues. Doses of chemotherapy that

Table 3 | **An example of optimal scheduling of cytotoxic and cytostatic therapy***

| Treatment | Advantages | Disadvantages |
|---|---|---|
| Chemotherapy followed by hormonal agent | No inhibition of cycle-dependent killing by chemotherapy | Delayed treatment with active hormonal agent. No inhibition of repopulation between cycles of chemotherapy |
| Chemotherapy and hormonal agent given concurrently | Early use of two active therapies. Inhibition of repopulation between cycles of chemotherapy | Inhibition of proliferation by hormonal agent might reduce cycle-dependent killing by chemotherapy |
| Short-acting hormonal agent given between cycles of chemotherapy and stopped before next cycle | Early use of two active therapies. Inhibition of repopulation between cycles of chemotherapy. No inhibition of cycle-dependent killing by chemotherapy | |

*Cytotoxic chemotherapy and tumour-selective cytostatic hormonal therapy options for adjuvant treatment of patients with hormone-receptor-positive breast cancer.

are tolerated during radiation are small, and their main effect might be to inhibit the repopulation of tumour cells[88,89]; if so, benefit is likely to occur when repopulation is faster in the tumour than in the dose-limiting normal tissues in the radiation field. Randomized trials for head and neck cancer have shown that concurrent chemotherapy can overcome the effects of prolonging the overall treatment time when planned treatment gaps are introduced[90,91]. Although radiation and concurrent chemotherapy is standard treatment for some types of cancer, toxic reactions prevent many patients from completing their chemotherapy[92], especially near the end of radiotherapy when repopulation is most likely to occur[89]. Encouraging results have been reported from Phase I and II studies that have evaluated concurrent chemotherapy delivered during the last part of the radiotherapy schedule[93–95].

***Use of cytostatic molecular-targeted agents.*** Repopulation probably depends on the activation of signalling pathways that stimulate the proliferation of tumour cells, and many molecular-targeted agents have been developed that inhibit these pathways. Examples of molecular-targeted agents include small molecule inhibitors and antibodies against specific proliferation pathway proteins. Some agents are approved for the systemic treatment of cancer and many others are in clinical trials. It is logical to evaluate the use of these agents during radiotherapy, or between courses of chemotherapy, with the goals of inhibiting the repopulation of tumour cells and improving the outcome of therapy.

Because signalling from the EGFR might be stimulated by radiation, repopulation might be inhibited by agents that bind to the EGFR or to the tyrosine kinase part of the receptor (EGFR–TK). Several single-arm studies have investigated EGFR inhibitors, including gefitinib, erlotinib, and the monoclonal antibody cetuximab. These results have been promising for tumour control, but also increased normal tissue toxicity. The ideal targeted agent enhances radiation response but has independent and limited side-effects, because acute normal tissue toxicities are at or near clinically acceptable tolerance levels for radiotherapy alone. Preliminary results of a Phase III study of cetuximab combined with radiotherapy for head and neck cancers demonstrated an improvement in locoregional control and survival compared with radiation alone, without significantly increasing acute mucosal toxicity[96]. Several molecular agents that target the MAPK pathway have been found to modulate radiosensitivity in preclinical studies and are in Phase I/II trials with radiation[97].

The potential use of tumour-selective cytostatic therapy to inhibit repopulation between courses of chemotherapy is illustrated in TABLE 3 by the example of scheduling of adjuvant chemotherapy (cytotoxic) and hormonal therapy (primarily cytostatic) after surgery for patients with hormone-receptor positive breast cancer. A logical strategy would use a short-acting cytostatic agent between courses of chemotherapy to inhibit the repopulation of tumour cells and to stop it before the next cycle so that cells can resume proliferation and be maximally sensitive to cytotoxic drugs. Hormonal agents provide an ideal tumour-specific strategy because the bone marrow, which is the most important organ that limits the dose and frequency of chemotherapy, is not affected. By modelling this concept, we have shown that the intermittent administration of the short-acting anti-oestrogen arzoxifene between treatments of hormone-responsive breast cancer cells with 5-fluorouracil or methotrexate could lead to a 100-fold reduction in the survival of clonogenic cells after two courses of treatment with chemotherapy[98].

The rapamycin analogue CCI-779 is a cytostatic agent that is particularly active against tumours with mutation of the tumour suppressor gene phosphatase and tensin homologue (*PTEN*). Mutations of *PTEN* are found in many human tumours, including prostate cancers[99,100]. We have investigated the use of CCI-779 in xenografts generated from the human prostate cancer cell lines PC-3 (mutant *PTEN*) and DU-145 (wild-type *PTEN*). CCI-779 caused marked cytostatic effects against PC-3 xenografts, and enhanced the effect of docetaxel when given between weekly courses of treatment[101].

Molecular-targeted agents are primarily cytostatic, so that tumour response (shrinkage) is relatively rare, and when it occurs, is usually delayed. In some clinical trials, these agents have been given concurrently and continuously with chemotherapy. The results of

INTENSITY-MODULATED RADIATION THERAPY
A form of 3-dimensional conformal radiotherapy. In this advanced radiotherapy technique, multiple radiation beams of varying intensity are used to 'shape' the radiation dose to encompass specified target volumes, while limiting the dose to normal tissues.

most trials have been disappointing. As illustrated in TABLE 3, this schedule is not logical, as the cytostatic effects of molecular-targeted agents might render tumour cells less sensitive to cycle-active chemotherapy. We suggest a new generation of clinical trials, in which cytostatic agents are given between courses of chemotherapy to inhibit repopulation, with the stopping of such treatment before the next round of chemotherapy to allow cells to re-enter the cycle and regain sensitivity to cycle-active drugs.

## Future directions

Repopulation during fractionated radiotherapy has long been recognized as an important cause of treatment failure. Clinical trials are being used to evaluate strategies to inhibit the process, and a promising approach is the use of molecular-targeted agents to selectively inhibit the proliferation of tumour cells during radiation treatment. Laboratory-based experiments and clinical trials should seek to identify tumour-selective cytostatic agents that are effective, but which do not interact with radiation to increase normal tissue toxicity. Combining such agents with conformal or INTENSITY-MODULATED RADIATION THERAPY, which limit the irradiation of normal tissues, might reduce the toxicity of combined therapies. Predictive assays, based on the molecular profiling of tumours, should seek tumour-specific strategies to inhibit repopulation.

Less is known about repopulation during chemotherapy, and priority should be given to experimental and clinical studies of the process, and of underlying mechanisms. As for radiotherapy, molecular-targeted agents offer opportunities to combine cytostatic tumour-selective therapy with chemotherapy. The optimal combination of such agents will require careful scheduling. Giving the patient the cytostatic agent continuously and concurrently is unlikely to be optimal, and new trials should investigate the scheduling of molecular-targeted agents between courses of chemotherapy.


1. Kummermehr, J. C. Tumour stem cells — the evidence and the ambiguity. *Acta Oncol.* **40**, 981–988 (2001).
2. Withers, H. R., Taylor, J. M. & Maciejewski, B. The hazard of accelerated tumour clonogen repopulation during radiotherapy. *Acta Oncol.* **27**, 131–146 (1988).
**This paper demonstrated reduced local control of head and neck cancer using radiotherapy with increase in overall treatment time; it was the first paper to demonstrate the clinical significance of accelerated repopulation.**
3. Malaise, E. & Tubiana, M. Growth of the cells of an experimental irradiated fibrosarcoma in the C3H mouse. *Hebd. Seances Acad. Sci., Ser. D, Sci. Nat.* **263**, 292–295 (1966) (in French).
4. Szczepanski, L. & Trott, K. R. Post-irradiation proliferation kinetics of a serially transplanted murine adenocarcinoma. *Br. J. Radiol.* **48**, 200–208 (1975).
5. Jung, H., Kruger, H. J., Brammer, I., Zywietz, F. & Beck-Bornholdt, H. P. Cell population kinetics of the rhabdomyosarcoma R1H of the rat after single doses of X-rays. *Int. J. Radiat. Biol.* **57**, 567–589 (1990).
6. Beck-Bornholdt, H. P., Omniczynski, M., Theis, E., Vogler, H. & Wurschmidt, F. Influence of treatment time on the response of rat rhabdomyosarcoma R1H to fractionated irradiation. *Acta Oncol.* **30**, 57–63 (1991).
7. Raabe, A. *et al.* Influence of dose per fraction and overall treatment time on the response of pulmonary micrometastases of the R1H-tumour to fractionated irradiation. *Radiother. Oncol.* **56**, 259–264 (2000).
8. Beck-Bornholdt, H. P. Should tumours be clamped in radiobiological fractionation experiments? *Int. J. Radiat. Oncol. Biol. Phys.* **21**, 675–682 (1991).
9. Milas, L., Yamada, S., Hunter, N., Guttenberger, R. & Thames, H. D. Changes in $TCD_{50}$ as a measure of clonogen doubling time in irradiated and unirradiated tumours. *Int. J. Radiat. Oncol. Biol. Phys.* **21**, 1195–1202 (1991).
10. Begg, A. C., Hofland, I. & Kummermehr, J. Tumour cell repopulation during fractionated radiotherapy: correlation between flow cytometric and radiobiological data in three murine tumours. *Eur. J. Cancer* **27**, 537–543 (1991).
11. Speke, A. K. & Hill, R. P. Repopulation kinetics during fractionated irradiation and the relationship to the potential doubling time, Tpot. *Int. J. Radiat. Oncol. Biol. Phys.* **31**, 847–856 (1995).
12. Suit, H. D., Howes, A. E. & Hunter, N. Dependence of response of a C3H mammary carcinoma to fractionated irradiation on fractionation number and intertreatment interval. *Radiat. Res.* **72**, 440–454 (1977).
13. Petersen, C. *et al.* Repopulation of FaDu human squamous cell carcinoma during fractionated radiotherapy correlates with reoxygenation. *Int. J. Radiat. Oncol. Biol. Phys.* **51**, 483–493 (2001).
14. Speke, A. K. & Hill, R. P. The effects of clamping and reoxygenation on repopulation during fractionated irradiation. *Int. J. Radiat. Oncol. Biol. Phys.* **31**, 857–863 (1995).
15. Hessel, F. *et al.* Repopulation of moderately well-differentiated and keratinizing GL human squamous cell carcinomas growing in nude mice. *Int. J. Radiat. Oncol. Biol. Phys.* **58**, 510–518 (2004).
16. Allam, A. *et al.* The effect of the overall treatment time of fractionated irradiation on the tumour control probability of a human soft tissue sarcoma xenograft in nude mice. *Int. J. Radiat. Oncol. Biol. Phys.* **32**, 105–111 (1995).
17. Sham, E. & Durand, R. E. Cell kinetics and repopulation parameters of irradiated xenograft tumours in SCID mice: comparison of two dose-fractionation regimens. *Eur. J. Cancer* **35**, 850–858 (1999).
18. Rofstad, E. K. Repopulation between radiation fractions in human melanoma xenografts. *Int. J. Radiat. Oncol. Biol. Phys.* **23**, 63–68 (1992).
19. Bentzen, S. M. & Thames, H. D. Clinical evidence for tumour clonogen regeneration: interpretations of the data. *Radiother. Oncol.* **22**, 161–166 (1991).
20. Withers, H. R. *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.* **33**, 549–562 (1995).
21. Begg, A. C. *et al.* The predictive value of cell kinetic measurements in a European trial of accelerated fractionation in advanced head and neck tumours: an interim report. *Int. J. Radiat. Oncol. Biol. Phys.* **19**, 1449–1453 (1990).
22. Maciejewski, B. & Majewski, S. Dose fractionation and tumour repopulation in radiotherapy for bladder cancer. *Radiother. Oncol.* **21**, 163–170 (1991).
23. Barton, M. B., Keane, T. J., Gadalla, T. & Maki, E. The effect of treatment time and treatment interruption on tumour control following radical radiotherapy of laryngeal cancer. *Radiother. Oncol.* **23**, 137–143 (1992).
24. Fyles, A., Keane, T. J., Barton, M. & Simm, J. The effect of treatment duration in the local control of cervix cancer. *Radiother. Oncol.* **25**, 273–279 (1992).
25. Tarnawski, R. *et al.* How fast is repopulation of tumour cells during the treatment gap? *Int. J. Radiat. Oncol. Biol. Phys.* **54**, 229–236 (2002).
26. Withers, H. R. & Peters, L. J. Transmutability of dose and time. Commentary on the first report of RTOG 90003 (K. K. FU *et al.*). *Int. J. Radiat. Oncol. Biol. Phys.* **48**, 1–2 (2000).
27. Fu, K. K. *et al.* A Radiation Therapy Oncology Group (RTOG) phase III randomized study to compare hyperfractionation and two variants of accelerated fractionation to standard fractionation radiotherapy for head and neck squamous cell carcinomas: first report of RTOG 9003. *Int. J. Radiat. Oncol. Biol. Phys.* **48**, 7–16 (2000).
**A report of a large randomized clinical trial that demonstrated improved locoregional control of head and neck squamous cell carcinomas with radiotherapy delivered by accelerated fractionation compared with conventional fractionation.**
28. Suwinski, R. *et al.* Time factor in postoperative radiotherapy: a multivariate locoregional control analysis in 868 patients. *Int. J. Radiat. Oncol. Biol. Phys.* **56**, 399–412 (2003).
29. Stephens, T. C. & Peacock, J. H. Tumour volume response, initial cell kill and cellular repopulation in B16 melanoma treated with cyclophosphamide and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. *Br. J. Cancer* **36**, 313–321 (1977).
30. Rosenblum, M. L., Knebel, K. D., Vasquez, D. A. & Wilson, C. B. *In vivo* clonogenic tumour cell kinetics following 1,3-bis(2-chloroethyl)-1-nitrosourea brain tumour therapy. *Cancer Res.* **36**, 3718–3725 (1976).
31. Rosenblum, M. L., Gerosa, M. A., Dougherty, D. V. & Wilson, C. B. Improved treatment of a brain-tumour model. Part 1: Advantages of single- over multiple-dose BCNU schedules. *J. Neurosurg.* **58**, 177–182 (1983).
32. Milas, L. *et al.* Dynamics of tumour cell clonogen repopulation in a murine sarcoma treated with cyclophosphamide. *Radiother. Oncol.* **30**, 247–253 (1994).
33. Wu, L. & Tannock, I. F. Repopulation in murine breast tumours during and after sequential treatments with cyclophosphamide and 5-fluorouracil. *Cancer Res.* **63**, 2134–2138 (2003).
34. Durand, R. E. & Vanderbyl, S. L. Tumour resistance to therapy: a genetic or kinetic problem? *Cancer Commun.* **1**, 277–283 (1989).
35. Durand, R. E. Multicell spheroids as a model for cell kinetic studies. *Cell Tissue Kinet.* **23**, 141–159 (1990).
36. Bourhis, J. *et al.* Rapid tumour cell proliferation after induction chemotherapy in oropharyngeal cancer. *Laryngoscope* **104**, 468–472 (1994).
37. Davis, A. J., Chapman, W., Hedley, D. W., Oza, A. M. & Tannock, I. F. Assessment of tumour cell repopulation after chemotherapy for advanced ovarian cancer: pilot study. *Cytometry A* **51**, 1–6 (2003).
38. Steel, G. G. *Growth Kinetics of Tumours: Cell Population Kinetics in Relation to the Growth and Treatment of Cancer* (Clarendon, Oxford, 1977).
39. Tannock, I. F. The relation between cell proliferation and the vascular system in a transplanted mouse mammary tumour. *Br. J. Cancer* **22**, 258–273 (1968).
40. Tannock, I. F. Population kinetics of carcinoma cells, capillary endothelial cells, and fibroblasts in a transplanted mouse mammary tumour. *Cancer Res.* **30**, 2470–2476 (1970).
41. Hirst, D. G., Denekamp, J. & Hobson, B. Proliferation kinetics of endothelial and tumour cells in three mouse mammary carcinomas. *Cell Tissue Kinet.* **15**, 251–261 (1982).
42. Fowler, J. F. Rapid repopulation in radiotherapy: a debate on mechanism. The phantom of tumour treatment — continually rapid proliferation unmasked. *Radiother. Oncol.* **22**, 156–158 (1991).
43. Kummermehr, J. & Trott, K. R. in *Stem cells* (ed. Potten, C. S.) 363–399 (Academic, London, 1997).
44. Dorr, W. Three A's of repopulation during fractionated irradiation of squamous epithelia: asymmetry loss, acceleration of stem-cell divisions and abortive divisions. *Int. J. Radiat. Biol.* **72**, 635–643 (1997).

45. Denekamp, J. The change in the rate of repopulation during multifraction irradiation of mouse skin. *Br. J. Radiol*. **45**, 801 (1972).
46. Denekamp, J. Changes in the rate of repopulation during multifraction irradiation of mouse skin. *Br. J. Radiol*. **46**, 381–387 (1973).
47. Petersen, C. *et al.* Proliferation and micromilieu during fractionated irradiation of human FaDu squamous cell carcinoma in nude mice. *Int. J. Radiat. Biol.* **79**, 469–477 (2003).
48. Balaban, N. *et al.* The effect of ionizing radiation on signal transduction: antibodies to EGF receptor sensitize A431 cells to radiation. *Biochim. Biophys. Acta* **1314**, 147–156 (1996).
49. Schmidt-Ullrich, R. K. *et al.* Molecular mechanisms of radiation-induced accelerated repopulation. *Radiat. Oncol. Investig.* **7**, 321–330 (1999).
50. Carter, S. *et al.* Inhibition of the mitogen activated protein (MAP) kinase cascade potentiates cell killing by low dose ionizing radiation in A431 human squamous carcinoma cells. *Oncogene* **16**, 2787–2796 (1998).
51. Kavanagh, B. D., Dent, P., Schmidt-Ullrich, R. K., Chen, P. & Mikkelsen, R. B. Calcium-dependent stimulation of mitogen-activated protein kinase activity in A431 cells by low doses of ionizing radiation. *Radiat. Res.* **149**, 579–587 (1998).
52. Bowers, G. *et al.* The relative role of ErbB1–4 receptor tyrosine kinases in radiation signal transduction responses of human carcinoma cells. *Oncogene* **20**, 1388–1397 (2001).
53. Schmidt-Ullrich, R. K. *et al.* Radiation-induced proliferation of the human A431 squamous carcinoma cells is dependent on EGFR tyrosine phosphorylation. *Oncogene* **15**, 1191–1197 (1997).
**This study demonstrated that radiation-induced proliferation of human mammary carcinoma (AG1478) cells requires activation of EGFR; it indicates that accelerated repopulation during a course of ionising radiation might be mediated through the EGFR pathway.**
54. Akimoto, T. *et al.* Inverse relationship between epidermal growth factor receptor expression and radiocurability of murine carcinomas. *Clin. Cancer Res.* **5**, 2884–2890 (1999).
55. Milas, L. *et al.* Relationship between cyclin D1 expression and poor radioresponse of murine carcinomas. *Int. J. Radiat. Oncol. Biol. Phys.* **52**, 514–521 (2002).
56. Grandis J. R., *et al*. Levels of TGF-α and EGFR protein in head and neck squamous cell carinoma and patient survival. *J. Natl Cancer Inst.* **90**, 824–832 (1998).
57. Ang, K. K. *et al.* Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. *Cancer Res.* **62**, 7350–7356 (2002).
58. Eriksen, J. G., Steiniche, T., Askaa, J., Alsner, J. & Overgaard, J. The prognostic value of epidermal growth factor receptor is related to tumour differentiation and the overall treatment time of radiotherapy in squamous cell carcinomas of the head and neck. *Int. J. Radiat. Oncol. Biol. Phys.* **58**, 561–566 (2004).
59. Norton, L. & Simon, R. Tumour size, sensitivity to therapy, and design of treatment schedules. *Cancer Treat. Rep.* **61**, 1307–1317 (1977).
**This paper provided an early model of tumour regrowth based on the application of the Gompertz equation. Although it did not use the term 'repopulation' the model is consistent with accelerated repopulation after cancer treatment.**
60. Norton, L. & Simon, R. The Norton-Simon hypothesis revisited. *Cancer Treat. Rep*. **70**, 163–169 (1986).
61. Davis, A. J. & Tannock, J. F. Repopulation of tumour cells between cycles of chemotherapy: a neglected factor. *Lancet Oncol.* **1**, 86–93 (2000).
**This paper provided models for the effect on tumour volume of repopulation between courses of chemotherapy. It showed that the common clinical experience of initial tumour response followed by regrowth during repeated courses of chemotherapy can be explained by accelerating repopulation.**
62. Withers, H. R. in *Radiation Research: A Twentieth Century Perspective*, Vol. II Congress Proceedings (ed. Dewey, W. C.) 26–31 (Academic, New York, 1991).
63. Freyer, J. P. & Sutherland, R. M. Proliferative and clonogenic heterogeneity of cells from EMT6/Ro multicellular spheroids induced by the glucose and oxygen supply. *Cancer Res.* **46**, 3513–3520 (1986).
64. Bredel-Geissler, A., Karbach, U., Walenta, S., Vollrath, L. & Mueller-Klieser, W. Proliferation-associated oxygen consumption and morphology of tumour cells in monolayer and spheroid culture. *J. Cell. Physiol*. **153**, 44–52 (1992).
65. Durand, R. E. Distribution and activity of antineoplastic drugs in a tumour model. *J. Natl Cancer Inst.* **81**, 146–152 (1989).
66. Lankelma, J. *et al.* Doxorubicin gradients in human breast cancer. *Clin. Cancer Res.* **5**, 1703–1707 (1703).
67. Jain, R. K. Delivery of molecular and cellular medicine to solid tumours. *Adv. Drug Deliv. Rev.* **46**, 149–168 (2001).
68. Tannock, I. F. Tumour physiology and drug resistance. *Cancer Metastasis Rev.* **20**, 123–132 (2001).
69. Tannock, I. F., Lee, C. M., Tunggal, J. K., Cowan, D. S. & Egorin, M. J. Limited penetration of anticancer drugs through tumour tissue: a potential cause of resistance of solid tumours to chemotherapy. *Clin. Cancer Res.* **8**, 878–8784 (2002).
70. Huxham, L. A., Kyle, A. H., Baker, J. H., Nykilchuk, L. K. & Minchinton, A. I. Microregional effects of gemcitabine in HCT-116 xenografts. *Cancer Res.* **64**, 6537–6541 (2004).
**This study showed that repopulation occurred predominantly from distal cells that had a low rate of proliferation in the untreated tumour.**
71. Withers, H. R. Biologic basis for altered fractionation schemes. *Cancer* **55**, 2086–2095 (1985).
**This paper discusses the radiobiological principles that underlie the choice of an accelerated fractionation radiotherapy schedule.**
72. Peters, L. J., Ang, K. K. & Thames, H. D., Jr. Accelerated fractionation in the radiation treatment of head and neck cancer. A critical comparison of different strategies. *Acta Oncol.* **27**, 185–194 (1988).
73. Norin, T. *et al.* Conventional and superfractionated radiation therapy in Burkitt's lymphoma. *Acta Radiol. Ther. Phys. Biol.* **10**, 545–557 (1971).
74. Bourhis, J. *et al.* Meta-analysis of conventional vs altered fractionated radiotherapy in head and neck squamous cell carcinoma (HNSCC): final analysis. *Int. J. Radiat. Oncol. Biol. Phys.* **60**, S190–S191 (2004).
75. Overgaard, J. *et al.* Five compared with six fractions per week of conventional radiotherapy of squamous-cell carcinoma of head and neck: DAHANCA 6 and 7 randomized controlled trial. *Lancet* **362**, 933–940 (2003).
76. Skladowski, K., Law, M. G., Maciejewski, B. & Steel, G. G. Planned and unplanned gaps in radiotherapy: the importance of gap position and gap duration. *Radiother. Oncol.* **30**, 109–120 (1994).
77. Poulsen, M. G. *et al.* A randomized trial of accelerated and conventional radiotherapy for stage III and IV squamous carcinoma of the head and neck: a Trans-Tasman Radiation Oncology Group Study. *Radiother. Oncol.* **60**, 113–122 (2001).
78. Dische, S. *et al.* A randomized multicentre trial of CHART versus conventional radiotherapy in head and neck cancer. *Radiother. Oncol.* **44**, 123–136 (1997).
79. Saunders, M. *et al.* (and on behalf of the CHART Steering Committee) Continuous hyperfractionated accelerated radiotherapy (CHART) versus conventional radiotherapy in non-small-cell lung cancer: a randomized multicentre trial. CHART Steering Committee. *Lancet* **350**, 161–165 (1997).
80. Saunders, M. *et al.* (and on behalf of the CHART Steering Committee) Continuous, hyperfractionated, accelerated radiotherapy (CHART) versus conventional radiotherapy in non-small cell lung cancer: mature data from the randomized multicentre trial. *Radiother. Oncol.* **52**, 137–148 (1999).
81. Citron, M. L. *et al.* Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of Intergroup Trial C9741/Cancer and Leukemia Group B Trial 9741. *J. Clin. Oncol.* **21**, 1431–1439 (2003).
**This report of a large randomized trial of adjuvant chemotherapy for patients with breast cancer showed improved survival for those receiving dose-dense chemotherapy delivered at two-week intervals with growth factor support, compared with those receiving standard three-week scheduling.**
82. Pfreundschuh, M. *et al.* Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of elderly patients with aggressive lymphomas: results of the NHL-B2 trial of the DSHNHL. *Blood* **104**, 634–641 (2004).
83. Sternberg, C. N. *et al.* Randomized phase III trial of high-dose-intensity methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) chemotherapy and recombinant human granulocyte colony-stimulating factor versus classic MVAC in advanced urothelial tract tumours: European Organization for Research and Treatment of Cancer Protocol no. 30924. *J. Clin. Oncol.* **19**, 2638–2646 (2001).
84. Baldini, E. *et al.* Accelerated versus standard cyclophosphamide, epirubicin and 5-fluorouracil or cyclophosphamide, methotrexate and 5-fluorouracil: a randomized phase III trial in locally advanced breast cancer. *Ann. Oncol.* **14**, 227–232 (2003).
85. Browman, G. P. *et al.* Choosing a concomitant chemotherapy and radiotherapy regimen for squamous cell head and neck cancer: A systematic review of the published literature with subgroup analysis. *Head Neck* **23**, 579–589 (2001).
86. Bourhis, J. & Pignon, J. P. Meta-analyses in head and neck squamous cell carcinoma. What is the role of chemotherapy? *Hematol. Oncol. Clin. North Am.* **13**, 769–775, vii (1999).
87. Eifel, P. J. *et al.* Pelvic irradiation with concurrent chemotherapy versus pelvic and para-aortic irradiation for high-risk cervical cancer: an update of Radiation Therapy Oncology Group Trial (RTOG) 90-01. *J. Clin. Oncol.* **22**, 872–880 (2004).
88. Tannock, I. F. Treatment of cancer with radiation and drugs. *J. Clin. Oncol.* **14**, 3156–3174 (1996).
89. Peters, L. J. & Withers, H. R. Applying radiobiological principles to combined modality treatment of head and neck cancer — the time factor. *Int. J. Radiat. Oncol. Biol. Phys.* **39**, 831–836 (1997).
90. Wendt, T. G. *et al.* Simultaneous radiochemotherapy versus radiotherapy alone in advanced head and neck cancer: a randomized multicenter study. *J. Clin. Oncol.* **16**, 1318–1324 (1998).
91. Keane, T. J. *et al.* A randomized trial of radiation therapy compared to split course radiation therapy combined with mitomycin C and 5 fluorouracil as initial treatment for advanced laryngeal and hypopharyngeal squamous carcinoma. *Int. J. Radiat. Oncol. Biol. Phys.* **25**, 613–618 (1993).
92. Furuse, K. *et al.* Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small-cell lung cancer. *J. Clin. Oncol.* **17**, 2692–2699 (1999).
93. Rischin, D. *et al.* Tirapazamine, cisplatin, and radiation versus fluorouracil, cisplatin, and radiation in patients with locally advanced head and neck cancer: A randomized phase II trial of the Trans-Tasman Radiation Oncology Group (TROG 98.02). *J. Clin. Oncol.* **23**, 79–87 (2005).
94. Corry, J. *et al.* Radiation with concurrent late chemotherapy intensification ('chemoboost') for locally advanced head and neck cancer. *Radiother. Oncol.* **54**, 123–127 (2000).
95. Garden, A. S. *et al.* Preliminary results of Radiation Therapy Oncology Group 97-03: a randomized phase II trial of concurrent radiation and chemotherapy for advanced squamous cell carcinomas of the head and neck. *J. Clin. Oncol.* **22**, 2856–2864 (2004).
96. Bonner, J. A. *et al.* Cetuximab prolongs survival in patients with locoregionally advanced squamous cell carcinoma of head and neck: A phase III study of high dose radiation therapy with or without cetuximab. *J. Clin. Oncol.* **22**, 5507 (2004).
97. Ma, B. B., Bristow, R. G., Kim, J. & Siu, L. L. Combined-modality treatment of solid tumours using radiotherapy and molecular targeted agents. *J. Clin. Oncol.* **21**, 2760–2776 (2003).
98. Wu, L. & Tannock, I. F. Selective estrogen receptor modulators as inhibitors of repopulation of human breast cancer cell lines after chemotherapy. *Clin. Cancer Res.* **9**, 4614–4618 (2003).
99. Neshat, M. S. *et al.* Enhanced sensitivity of PTEN-deficient tumours to inhibition of FRAP/mTOR. *Proc. Natl Acad. Sci. USA* **98**, 10314–10319 (2001).
100. Visakorpi, T. The molecular genetics of prostate cancer. *Urology* **62**, 3–10 (2003).
101. Wu, H., Birle, D. C. & Tannock, I. F. Effects of the mammalian target of rapamycin inhibitor CCI-779 used alone or with chemotherapy on human prostate cancer cells and xenografts. *Cancer Res.* **65**, 2825–2831 (2005).



Competing interests statement
The authors declare no competing financial interests.


## Online links

**DATABASES**

**The following terms in this article are linked online to:**

**Cancer.gov:** http://www.cancer.gov
bladder cancer | breast cancer | cervical cancer | head and neck cancer | non-Hodgkin's lymphoma | non-small cell lung cancer | oesophageal cancer | oropharyngeal cancer | ovarian cancer | laryngeal cancer | prostate cancer

**FURTHER INFORMATION**

**Tannock laboratory:** http://medbio.utoronto.ca/faculty/tannock.html
**Radiation Therapy Oncology Group:** http://www.rtog.org
**Access to this interactive links box is free online.**