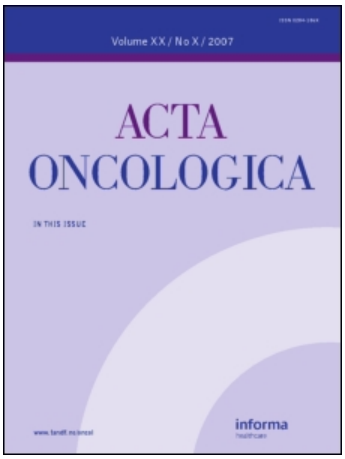

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Azidothymidine (AZT) as a Potential Modifier of Radiation Response in Vitro

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AZIDOTHYMIDINE (AZT) AS A POTENTIAL MODIFIER OF RADIATION RESPONSE IN VITRO

MARIE-LAURE COPACEANU, PHILIPPE A. COUCKE, ELIANE COTTIN, NICOLAS PASCHOUD and RENE-OLIVIER MIRIMANOFF

The potential effect of AZT as a thymidine analogue on radiation response in vitro was investigated. Two human cell lines (WiDr and HeLa) were used. The effect of 10 μM AZT on exponentially growing cells was studied after different exposure times (24, 48 and 72 h). The surviving fraction (clonogenic assay) or metabolic activity (MTT assay) after irradiation of AZT-exposed cells, was compared to unexposed irradiated controls. Flow cytometry was used to assess the cell-cycle effect of pre-exposure of exponentially growing cells to AZT. AZT had a radioprotective effect for all experimental time points as far as WiDr was concerned. For HeLa the effect was significant at 24 h. Cell-cycle analysis showed a significant accumulation in S-phase at 72 h for WiDr. For HeLa there was a significant accumulation in S-phase at 48 h. We conclude that under the reported experimental conditions, AZT as a thymidine analogue seems to reduce the cytotoxic effect of irradiation.

Since the discovery in 1960 of the radiosensitizing effect of nucleoside analogues such as 5-bromodeoxyuridine (BrdUrd) and 5-iododeoxyuridine (IdUrd) (1), and other compounds such as 5-chloro-2′-deoxycytidine (CldCyd) and 5-bromo-2′-deoxycytidine (BrdCyd) (2), extensive research has been carried out both in vitro and in vivo (3–13).

Azidothymidine (AZT, 3′-azido-3′-deoxythymidine, zidovudine), another thymidine analogue, is well known in clinical practice for its application in the treatment of AIDS and AIDS-related complex. This drug must first be activated to a 5′-triphosphate form, which exerts its antiretroviral activity at the level of reverse transcriptase (viral DNA polymerase). Azidothymidine inhibits this enzyme, essential for HIV replication, and is incorporated into viral DNA leading to chain termination (14–17). In patients suffering from AIDS, radiation therapy is often used as a treatment modality for various tumors. Therefore, we investigated the potential effect of AZT as a modifier of radiation response in vitro on two different human tumor cell lines (WiDr and HeLa). Two different techniques were compared: the clonogenic assay, and the MTT assay (measurement of viability). Flow cytometry was used in order to assess the influence of AZT on distribution of cells in different phases of cell cycle.

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Material and Methods

Chemicals and drugs. AZT was provided by the Wellcome Foundation Ltd (London, England). DMSO, MTT and Propidium Iodide were purchased from Sigma Chemie (Buchs, Switzerland). Pepsin was purchased from Merck AG (Switzerland). MTT was dissolved, at a concentration of 5 mg/ml in RPMI-1640 medium without phenol red, filtered and frozen for stockage (according to Sigma).

Cell culture. Cells were cultured in media and supplements purchased from Gibco BRL (Basel, Switzerland). Fetal calf serum was obtained from Fakola AG (Basel, Switzerland). The two cell lines were obtained from ATCC (Rockville, MD). The WiDr cell line, a human primary adenocarcinoma of the rectosigmoid colon, was maintained in Minimum Essential Medium with 0.85 GM/L

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$NAHCO_3$ supplemented with 10% fetal calf serum, 1% non-essential amino acids, 2 mM L-glutamine and 1% penicillin-streptomycin solution. WiDr has a 24-h doubling time and a PE of approximately 99%. The HeLa cell line, a human adenocarcinoma of the cervix, was maintained in Optimem 1 medium supplemented with 5% fetal calf serum and 1% penicillin-streptomycin solution. HeLa has a 15-h doubling time and a PE of approximately 98%. Cells were incubated in a humidified, 5% CO_2 atmosphere at 37°C. Cells were passaged twice weekly. Mycoplasma contamination was checked every 6 months. For the MTT assay, the two cell lines were maintained in RPMI-1640 medium (without L-glutamine and phenol red), supplemented with 10% fetal calf serum, 2mM L-glutamine and 1% penicillin-streptomycin solution. Phenol red was suppressed in the incubation medium (18). Overall, there was no phenol red in the medium used both for maintenance of cell line and experimental conditions for the MTT assay.

Irradiation. The cells were irradiated at room temperature with an Oris IBL 637 cesium source at a dose rate of 0.8 Gy/min. Doses from 1 to 10 Gy, with a 1 Gy dose increment, and 15 Gy were used.

AZT effect on cells. Exponentially growing WiDr and HeLa cells were seeded in 60 × 15-mm Falcon Primaria culture dishes with 5 ml of medium and allowed to incubate at 37°C for approximately 24 h before adding AZT. Medium was replaced every 24 h and completed by adding the appropriate amount of freshly prepared AZT in order to obtain a constant concentration of 10 μM of active drug. Lack of confluence was checked microscopically. All experiments were repeated thrice.

Clonogenic assay. The cells were trypsinised and resuspended in fresh medium. An appropriate number of cells were plated into 100 × 20 mm Falcon Primaria culture dishes containing 10 ml of medium. After cell attachment, dishes were irradiated (10 dose levels from 1 to 10 Gy). For each radiation dose, four dishes were utilized. An equivalent number was used in order to control baseline plating efficiency without irradiation both for controls and drug exposed cells. The counting of the clones was done manually 14 days after seeding. Clones of more than 50 cells were considered survivors. All clonogenic assays were repeated thrice. There was no significant effect of AZT on plating efficiency, at a concentration of 10 μM for the three incubation times (24, 48 and 72 h) on the two cell lines.

MTT assay. The original assay (19) was modified according to Denizot & Lang (18) and Twentyman & Luscombe (20). The cells were trypsinised and resuspended in fresh medium. The cells were seeded in a Falcon Primaria 96-well plate at an appropriate concentration according to each cell line. The assay was carried out in one half-area of the microtitre plate for the control and the other for the drug. After cell fixation, plates were irradiated (11 dose levels from 1 to 10 Gy and 15 Gy). The results were always normalized according to the baseline optical density obtained on an unirradiated microtitre plate. In order to eliminate the apparent resistant tail at high doses, due to the presence of doomed cells, the activity of surviving cells at 15 Gy was substracted from both treated and control values (21). The plates were incubated during 72 h. At this time point, both cell lines showed a lack of confluent growth. Moreover, cells should have divided at least three times in the absence of irradiation without a need for medium replacement. After 72 h the culture-medium was removed and the MTT assay performed. The plates were read at 570 nm using a SLT-SPECTRA II (Tecan AG, Hombrechtikon, Switzerland) plate recorder. The reference wavelength was set at 690 nm. The optical density reached a level of 0.8 to 1.0 which is considered optimal (22). Each experiment was repeated thrice.

Flow cytometry. Cells were prepared and exposed to AZT in the same experimental conditions as described for clonogenic and MTT assays. Then the cells were trypsinised, fixed in 70% ethanol and stored in the dark at 4°C until the day of analysis. Begg's method was used for cell preparation for flow cytometry on a FAC SCAN (Becton Dickinson) (23). The DNA content was measured to establish the cell cycle distribution. 15 000 events, acquired on List Mode, were analysed by 'Multiplus' (Phoenix Flow, San Diego, California). The approach was pioneered by Jett & Dean (24). The particular method used by MULTICYCLE is a nonlinear least squares fitting by a method described by Marquardt (25). This method required complex mathematical manipulations and successive iterations until the fit was optimised. Each experiment was repeated thrice.

Data analysis. Statistical analysis was carried out with the Statview software package on Macintosh SE/30 using a paired t-test (two-sided). Results were considered statistically significant if a p-value of less than 0.05 was reached. Standard errors were calculated but not plotted on the figures because they were in the range of the used symbols.

Results

Clonogenic assay. The results of this assay, aimed at estimating clonogenicity are summarized in Figs. 1 and 2 (WiDr and HeLa respectively). All observed survival data were referred to the corresponding baseline plating efficiency of unirradiated cells taking into account pre-exposure or not with AZT. The surviving fraction (SF) was calculated by comparing the plating efficiency of irradiated and AZT-exposed cells to irradiated controls. The AZT-effect on radiation response was investigated as a time dependent phenomenon. We did not investigate the concentration-effect of AZT, i.e. all experiments were performed at a 10-μM concentration. Ten micromolar was chosen, because this concentration was close to therapeutic values (26). Considering the survival curve, there was a

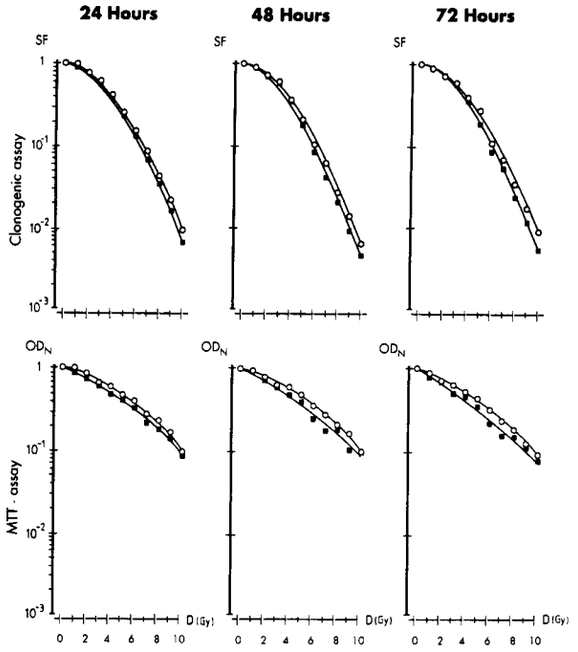

Fig. 1. Clonogenic and MTT assays for WiDr exponentially growing cells: comparison between irradiated controls and cells exposed to AZT prior to irradiation.
○: AZT; ■: Control.

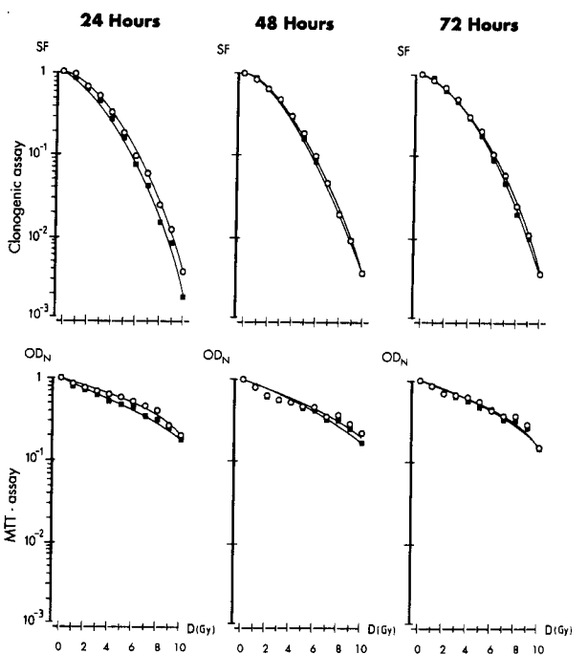

Fig. 2. Clonogenic and MTT assays for HeLa exponentially growing cells: comparison between irradiated controls and cells exposed to AZT prior to irradiation.
○: AZT; ■: Control.

consistent and statistically significant increase of survival of AZT-exposed WiDr cells at 24, 48 and 72 h compared to unexposed irradiated cells as illustrated in Fig. 1 and a decrease in the shoulder as indicated by the initial slope (alpha) of the survival curves for all of the exposure times investigated (see Table 1). Beta, however, was apparently not significantly changed by preincubation with AZT. The dose modifying factors at 30% survival level (DMF_{30}) are listed in Table 1. For the HeLa cell line, there was again a radioprotective effect observed at 24 h whereas the significance of this effect disappeared at 48 and 72 h. However, the trend toward an increased survival after AZT exposure was maintained. The alpha values and DMF_{30} are summarized in Table 1.

MTT assay. An overview of the results of the MTT assay, aimed at estimating viability, i.e. metabolic capacity to reduce the tetrazolium salt MTT, is shown in Figs. 1 and 2 according to each cell line. The optical density (OD) was corrected in function of the base-line optical density obtained without irradiation for AZT-exposed and unexposed cells respectively. Again, the AZT-effect on radiation response was investigated as a time-dependent phenomenon. We did not investigate the concentration effect. There was a consistent and statistically significant increase of viability of AZT exposed WiDr cells at 24, 48 and 72 h as illustrated on the 'OD versus dose' curves in Fig. 1. This significant decrease in the initial slope (iota: ι) occurred at all time points investigated (see Table 2). Theta (the final slope: τ), however, was apparently not significantly changed by preincubation with AZT. The DMF_{30} for WiDr are listed in Table 2.

In contrast to WiDr, the AZT effect on the HeLa cell line was only statistically significant at 24 h. However, at 48 and 72 h there was again a trend towards radioprotection as was the case with the clonogenic assay. The iota values and DMF_{30} are summarized in Table 2. The data

Table 1

Effects of AZT on intrinsic radiosensivity (α) and DMF_{30} (clonogenic assay)

Cell line	WiDr			HeLa		
Time of incubation	24 h	48 h	72 h	24 h	48 h	72 h
$\alpha_C \pm$ S.E.	0.104 ± 0.022	0.196 ± 0.019	0.150 ± 0.004	0.130 ± 0.034	0.159 ± 0.039	0.140 ± 0.021
$\alpha_{AZT} \pm$ S.E.	0.084 ± 0.030	0.139 ± 0.015	0.120 ± 0.012	0.115 ± 0.035	0.119 ± 0.033	0.078 ± 0.022
DMF_{30}	1.07	1.1	1.13	1.11	1.03	1.03

Table 2

Effect of AZT on metabolic activity after irradiation (ι) and $DMF_{30'}$ (MTT assay)

Cell line	WiDr			HeLa		
Time of incubation	24 h	48 h	72 h	24 h	48 h	72 h
$\iota_C \pm$ S.E.	0.127 ± 0.026	0.176 ± 0.026	0.225 ± 0.029	0.066 ± 0.033	0.085 ± 0.025	0.068 ± 0.028
$\iota_{AZT} \pm$ S.E.	0.085 ± 0.011	0.077 ± 0.025	0.119 ± 0.020	0.025 ± 0.017	0.035 ± 0.005	0.045 ± 0.012
$DMF_{30'}$	1.1	1.17	1.19	1.11	1.06	1.04

Table 3

Effects of AZT on the cell cycle distribution at different exposure times

Cell line	WiDr	HeLa	WiDr	HeLa	WiDr	HeLa
	G_0–$G_1 \pm$ S.E.		S $\pm$ S.E.		G_2–M $\pm$ S.E.	
C_{24}	57 ± 2	61 ± 1	32 ± 1	30 ± 1	11 ± 1	9 ± 0
AZT_{24}	53 ± 2	60 ± 2	36 ± 1	32 ± 2	11 ± 1	8 ± 1
P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
C_{48}	69 ± 0	72 ± 0	24 ± 0	20 ± 1	7 ± 0	8 ± 1
AZT_{48}	58 ± 6	66 ± 0	34 ± 5	26 ± 1	8 ± 1	8 ± 1
P	>0.05	<0.05	>0.05	<0.05	>0.05	>0.05
C_{72}	71 ± 0	70 ± 3	22 ± 1	25 ± 5	7 ± 1	5 ± 2
AZT_{72}	67 ± 1	67 ± 1	26 ± 1	25 ± 2	7 ± 1	8 ± 1
P	<0.05	>0.05	$=0.05$	>0.05	>0.05	>0.05

obtained by both experimental techniques (clonogenic and MTT assays) were consistent although there were quantitative differences.

Flow cytometry. Incubation of exponentially growing WiDr cells with AZT, resulted in an accumulation of cells in S-phase with a concomitant reduction of cells in G_0–G_1 Table 3). However, this accumulation in S-phase was only significant at 72 h. This reduction was also observed for HeLa, but was only significant at 48 h (Table 3). WiDr and HeLa cells were exposed to BrdUrd at a 10 μM concentration and there was a trend ($p < 0.10$) towards a reduction of S-phase fraction, especially at 48 and 72 h for WiDr and at 24 h for HeLa (data not shown).

Discussion

Nucleoside analogues, such as BrdUrd and IdUrd, are known to act as radiosensitizers (1, 3–6, 8 13) and have already been used in clinical trials (10, 27). The hypothesis concerning the radiosensitizing effect of halogenated pyrimidines is partly based on a fraudulent nucleoside concept. Thymidine analogues (BrdUrd and IdUrd) are phosphorylated (pyrimidine salvage pathway) and complete with native dTMP for subsequent phosphorylation and incorporation into DNA (1, 28–30). The radionsensitizing effect is dependent on the amount of thymidine replacement (31, 8, 5).

Based on these published data, we investigated the potential modifying effect of AZT, another thymidine analogue, which is extensively used in the clinical approach of AIDS-patients (17). AZT, more specifically its phosphorylated metabolite (AZT-5'-triphosphate), selectively inhibits viral replication. Its antiviral activity is based on inhibition of viral reverse transcriptase and chain termination by incorporation into viral DNA (14, 32).

More recently, AZT has been shown to be a modifier of chemotherapeutic response, especially for 5-fluorouracil and methotrexate. This requires incorporation of AZT in tumor cells (33, 35). The enhanced effect of cytotoxic drugs is based on inhibition of thymidine kinase (33–35), which is a key enzyme in the nucleotide salvage pathway. The enzymes of de novo and salvage nucleotide biosynthesis in cancer cells have been shown to be increased as compared to normal cells (36–37). Therefore, one might speculate on the selective activity of AZT as far as modulation of chemotherapy is concerned.

Radiation therapy as another cytotoxic modality might be modified in presence of inhibitors of nucleotide salvage pathway such as AZT. We investigated the effect of exposure of WiDr and HeLa cell lines to AZT prior to irradiation in exponential growth conditions. However, in both experimental conditions (i.e. clonogenic and MTT assays), AZT resulted in a radioprotective effect, which was significant for WiDr at all exposure times, and for HeLa at 24 h only.

The cell cycle analysis of AZT-effect on exponentially growing WiDr showed a small but significant accumulation of cells in S-phase at 72 h. The same trend, although not statistically significant, was observed at 24 and 48 h. In contrast to WiDr the accumulation in S-phase for HeLa cells was only significant at 48 h.

In general, cells are most radiosensitive in M and G_2 phase, and most resistant in late S-phase of the cell cycle (38). Our comparison with BrdUrd in the same experimental conditions (data not shown) demonstrated a differential effect on the S-phase. With BrdUrd, known as a radiosensitizer, there is a reduction of the number of cells in S, whereas with AZT there is an increase of proportion of cells in S. Although cell cycle redistribution as a mechanism of radioprotection is attractive, it is quite possible that other mechanisms are related to the radiation response after AZT exposure.

These data indicate a potential radioprotection effect of AZT. However, extrapolation of these in vitro data, to in vivo situations may be hazardous. This information should be considered cautiously and submitted to more extensive research prior to any definite conclusion about the potential hazard to irradiate tumors in patients with AIDS and AIDS-related complex.

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REFERENCES

1. Djordjevic B, Szybalsky W. Genetics of human cell lines. III. Incorporation of 5-bromo-and 5-iododeoxyuridine into the deoxyribonucleic acid of human cells and its effect on radiation sensitivity. J Exp Med 1960; 112: 509–31.
2. Lawrence TS, Davis MA. Selective radiosensitization and cytotoxicity of human melanoma cells using halogenated deoxycytidines and tetrahydrouridine. Int J Radiat Oncol Biol Phys 1989; 10: 1243–6.
3. Iliakis G, Kurtzman S, Pantelias G, Okayasu R. Mechanism of radiosensitization by halogenated pyrimidines: effect of BrdU on radiation induction of DNA and chromosome damage and its correlation with cell killing. Radiat Res 1989; 119: 286–304.
4. Speth PA, Kinsella TJ, Chang AE, et al. Iododeoxyuridine (IdUrd) incorporation into DNA of human hematopoietic cells, normal liver and hepatic metastases in man: as a radiosensitizer and as a marker for cell kinetic studies. Int J Radiat Oncol Biol Phys 1989; 16: 1247–50.
5. Phillips TL, Bodell WJ, Uhl V, Ross GY, Rasmussen J, Mitchell JB. Correlation of exposure time concentration and incorporation of IdUrd in V-79 cells with radiation response. Int J Radiat Oncol Biol Phys 1989; 16: 1251–5.
6. Ling LL, Ward JF. Radiosensitization of Chinese hamster V79 cells by bromodeoxyuridine substitution of thymidine: enhancement of radiation-induced toxicity and DNA strand break production by monofilar and bifilar substitution. Radiat Res 1990; 121: 76–83.
7. Fornace AJ, Dobson PP, Kinsella TJ. Enhancement of radiation damage in cellular DNA following unifilar substitution with iododeoxyuridine. Int J Radiat Oncol Biol Phys 1990; 18: 873–8.
8. Lawrence TS, Davis MA, Maybaum J, Stetson PL, Ensminger WD. The dependence of halogenated pyrimidine incorporation and radiosensitization on the duration of drug exposure. Int J Radiat Oncol Biol Phys 1990; 18: 1393–8.
9. Wirtanen GW, Wiley AL, Vermund H, Stephenson JA, Ansfield FJ. Intraarterial iododeoxyuridine infusion combined with irradiation. Am J Clin Oncol 1990; 13: 320–3.
10. Hegarty TJ, Thornton AF, Diaz RF, et al. Intra-arterial bromodeoxyuridine radiosensitization of malignant gliomas. Int J Radiat Oncol Biol Phys 1990; 19: 421–8.
11. Tishler RB, Geard CR. Low dose rate irradiation and halogenated pyrimidine effects on human cervical carcinoma cells. Int J Radiat Oncol Biol Phys 1991; 21: 975–82.
12. Wang Y, Iliakis G. Effects of 5′-iododeoxyuridine on the repair of radiation induced potentially lethal damage interphase chromatin breaks and DNA double strand breaks in chinese hamster ovary cells. Int J Radiat Oncol Biol Phys 1992; 23: 353–60.
13. Uhl V, Phillips TL, Ross GY, Bodell WJ, Rasmussen J. Iododeoxyuridine incorporation and radiosensitization in three human tumor cell lines. Int J Radiat Oncol Biol Phys 1992; 22: 489–94.
14. Furman PA, Fyfe JA, Clair MHS, et al. Phosphorylation of 3′-azido-3′-deoxythymidine and selective interaction of the 5′-triphosphate with human immunodeficiency virus reverse transcriptase. Proc Natl Acad Sci USA 1986; 83: 8333–7.
15. Fischl MA, Richman DD, Grieco MH, et al. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. N Engl J Med 1987; 317: 185–91.
16. Langtry HD, Campoli-Richards DM. Zidovudine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. Drugs 1989; 37: 408–50.
17. Yarchoan R, Mitsuya H, Myers CE, Broder S. Clinical Pharmacology of 3′-azido-2′, 3′-dideoxythymidine (zidovudine) and related dideoxynucleosides. N Engl J Med 1989; 321: 726–38.
18. Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 1986; 89: 271–7.
19. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55–63.
20. Twentyman PR, Luscombe M. A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. Br J Cancer 1987; 56: 279–85.
21. Begg AC, Mooren E. Rapid fluorescence-based assay for radiosensitivity and chemosensitivity testing in mammalian cells in vitro. Cancer Res 1989; 49: 565–9.
22. Ramsay J, Ward R, Bleehen NM. Radiosensitivity testing of human malignant gliomas. Int J Radiat Oncol Biol Phys 1992; 24: 675–80.
23. Begg AC, Hofland I, Moonen L, et al. The predictive value of cell kinetic measurements in a european trial of accelerated fractionation in advanced head and neck tumors: an interim report. Int J Radial Oncol Biol Phys 1990; 19: 1449–53.
24. Jett P, Dean J. Mathematical analysis of DNA distributions derived from flow micro-fluorimetry. Cell Biol 1974; 60: 523.
25. Marquardt DW. An algorithm for least-squares estimation of nonlinear parameters. Soc Ind Appl Math 1963; 11: 431–41.
26. Munch-Petersen B. Azidothymidine inhibits mitogen stimulated growth and DNA-repair in human peripheral lymphocytes. Biochem Biophys Res Commun 1988; 157: 1369–75.
27. Chang AE, Collins JM, Speth PAJ, et al. T.J., A phase 1 study of intraarterial iododeoxyuridine in patients with colorectal liver metastases. J Clin Oncol 1989; 7: 662–8.
28. Erikson RL, Szybalski W. Molecular radiobiology of human cell lines. III. Radiation-sensitizing properties of 5-iododeoxyuridine. Cancer Res 1963; 23: 122–30.
29. Erikson RL, Szybalski W. Molecular radiobiology of human cell lines. V. Comparative radiosensitizing properties of 5-halodeoxycytidines and 5-halodeoxyuridines. Radiat Res 1963; 20: 252–62.
30. Lett JT, Parkins G, Alexander P. Mechanisms of sensitization to x-rays of mammalian cells by 5-bromodeoxyuridine. Nature 1964; 203: 593–6.
31. Kinsella TJ. Radiosensitization and cell kinetics: clinical implications for S-phase specific radiosensitizers. ASCO Educational Book, 27th Annual Meeting 1991: 174–81.
32. Heidenreich O, Kruhoffer M, Grosse F, Eckstein F. Inhibition of human immunodeficiency virus 1 reverse transcriptase

by 3'-azidothymidine triphosphate. Eur J Biochem 1990; 192: 621–5.
33. Brunetti I, Falcone A, Calabresi P, Goulette FA, Darnowski JW. 5-Fluorouracil enhances azidothymidine cytotoxicity: In vitro, in vivo, and biochemical studies. Cancer Res 1990; 50: 4026–31.
34. Weber G, Nagai M, Prajda N, Nakamura H, Szekeres T, Olah E. AZT: a biochemical response modifier of methotrexate and 5-fluorouracil cytotoxicity in human ovarian and pancreatic carcinoma cells. Cancer Commun 1991; 3: 127–32.
35. Tosi P, Calabresi P, Goulette FA, Renaud CA, Darnowski JW. Azidothymidine-induced cytotoxicity and incorporation into DNA in the human colon tumor cell line HCT-8 is enhanced by methotrexate in vitro and in vivo. Cancer Res 1992; 52: 4069–73.
36. Denton JE, Lui MS, Aoki T, et al. Enzymology of pyrimidine and carbohydrate metabolism in human colon carcinomas. Cancer Res 1982; 42: 1176–83.
37. Weber G. Biochemical strategy of cancer cells and the design of chemotherapy: G.H.A. Clowes Memorial Lecture. Cancer Res 1983; 43: 3466–92.
38. Sinclair WK, Morton RA. X-ray sensitivity during the cell generation cycle of cultured Chinese hamster cells. Radiat Res 1966; 29: 450–74.