HD. In case a second-line chemotherapy is required, MOPP is probably not the best combination, since second haematological malignancies can develop.


1. DeVita VT Jr, Serpick AA, Carbone PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. *Ann Intern Med* 1970, **73**, 881–895.
2. Selby P, Patel P, Milan S, *et al.* ChlVPP combination chemotherapy for Hodgkins' disease: long term results. *Br J Cancer* 1990, **62**, 279–285.
3. Tucker MA, Coleman CN, Cox RS, Varghese A, Rosenberg SA. Risk of second malignancies after treatment for Hodgkin's disease. *N Engl J Med* 1988, **318**, 76–81.
4. Bonadonna G, Zucali R, Monfardini S, De Lena M, Uslenghi C. Combination chemotherapy for Hodgkin's disease with adriamycin, bleomycin, vinblastine and imidazole carboxamide versus MOPP. *Cancer* 1975, **36**, 252–259.
5. Taylor ME, McElwain TJ, Barrett A, Peckham MJ. Etoposide as a single agent in advanced relapsed lymphomas. A phase II study. *Cancer Chemother Pharmacol* 1982, **7**, 175–177.
6. Perren TJ, Selby PJ, Milan S, McElwain TJ. Etoposide and adriamycin containing combination chemotherapy (HOPE-Bleo) for relapsed Hodgkin's disease. *Br J Cancer* 1990, **61**, 919–923.
7. Richards MA, Waxman JH, Man T, *et al.* EVA treatment for recurrent or unresponsive Hodgkin's disease. *Cancer Chemother Pharmacol* 1986, **18**, 51–53.
8. Santoro A, Viviani P, Valagussa P, Bonfante V, Bonadonna G. CCNU, etoposide and prednimustine (CEP) in refractory Hodgkin's disease. *Seminars in Oncology* 1986, **13**, 23–26.
9. Geller RB, Vogelsang GB, Winjond JR, *et al.* Successful marrow transplantation for acute myelocytic leukemia following therapy for HD. *J Clin Oncol* 1988, **6**, 1558–1561.
10. Sargur M, Buckner CD, Appelbaum FR, *et al.* Marrow transplantation for acute non lymphocytic leukemia following therapy for HD. *J Clin Oncol* 1987, **5**, 731–734.
11. Ratain MJ, Kaminer LS, Bitran JD, *et al.* Acute nonlymphocytic leukemia following etoposide and cisplatin combination chemotherapy for advanced non-small-cell carcinoma of the lung. *Blood* 1987, **70**, 1412–1417.
12. Pedersen-Bjergaard J, Daugaard G, Hansen SW, Philip P, Larsen SO, Rorth M. Increased risk of myelodysplasic and leukemia after etoposide, cisplatin and bleomycin for germ cell tumors. *Lancet* 1991, **338**, 359–363.
13. Pui CH, Ribeiro RC, Hancock ML, *et al.* Acute myeloid leukemia in children treated with epipodophyllotoxins for acute lymphoblastic leukemia. *N Engl J Med* 1991, **325**, 1682–1687.
14. Mansi JL, Cunningham D, Selby PJ, McElwain TJ. VEEP: a new chemotherapy regimen for Hodgkin's disease. *Annals of Oncology* 1990, **1** (suppl.), 1.


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# Changes of Lymphocyte Subsets after Local Irradiation for Early Stage Breast Cancer and Seminoma Testis: Long-term Increase of Activated (HLA-DR+) T Cells and Decrease of "Naive" (CD4-CD45R) T Lymphocytes

**Dirk De Ruysscher, Mark Waer, Michel Vandeputte, Rita Aerts, Kris Vantongelen and Emmanuel van der Schueren**

**Blood lymphocyte subsets of early breast cancer patients and of men with stage I seminoma of the testis were studied up to 6 years after radiotherapy. Similar results were obtained in the two patient groups. After a temporary decrease, the CD4-w29 or "memory" T cells recovered completely, while the CD4-45R or "naive" T cells remained decreased up to 6 years after irradiation. The number of CD8 T lymphocytes did not change during or after treatment. Because of the decrease of a subset of CD4 cells, and the unchanged values of CD8 cells, the CD4/CD8 ratio decreased significantly after irradiation, and remained lower than before treatment up to 5–6 years after radiotherapy. The number of both HLA-DR positive CD4 and HLA-DR positive CD8 T cells ("activated" T cells) increased significantly after irradiation. The natural killer (NK) cells were not affected by treatment. We propose that the recovery of the CD4 cells is limited to the CD4-w29 ("memory") population because of thymic dysfunction in older humans. The impact of the observed immune modulation on the low susceptibility for infections after local irradiation, and on putative antitumour immune responses is discussed.**
*Eur J Cancer*, Vol. 28A, No. 10, pp. 1729–1734, 1992.

## INTRODUCTION

SEVERAL INVESTIGATORS have described changes in the lymphocyte populations after local radiotherapy in humans [1–4]. A lymphopenia and a decreased CD4/CD8 ratio were the most striking observations. To investigate in more detail the changes of lymphocyte subgroups after clinical radiotherapy, we initiated a study in irradiated stage I–II breast cancer and stage I seminoma testis patients. Patients with seminoma testis were included in the study to investigate the possible influence of sex (male), age (generally a young population), and location (thoracic vs. infradiaphragmatic fields) on the immune changes after radiotherapy. We not only wanted to verify the previously

described decreased CD4/CD8 ratio in patients, but we were also interested to see how other cell subpopulations were influenced by local radiotherapy. More specifically, we were interested in the recovery of CD4+45R+ lymphocytes, which are generally accepted as being unstimulated short-lived "naive" lymphocytes with limited self-renewal capacity and in the CD4+w29+lymphocytes which are "memory" T cells [5]. Our hypothesis was that following radiotherapy, the recovery of mainly "naive" T lymphocytes which are dependent on thymic production would be hampered in adult patients as the thymus is involuted at that age which may lead to a deficient function [6]. This would result in a decrease of the whole CD4 T-cell population. The CD8 cells which depend less on thymic production [7] would recover to normal levels. The breast cancer patients were studied both retro- and prospectively, the seminoma patients only retrospectively.

## PATIENTS AND METHODS

*Patients*

Data obtained from patients with seminoma of the testis came from a retrospective study. Staging was done according to the UICC (1987).

10 patients with pathological stage I pure seminoma of the testis, which were disease free for 5 years, were studied. All patients were in a good general condition (Karnofsky index 90–100%), had no other cancer, did not take drugs, and had no other disease. All were treated with inguinal orchiectomy, followed by irradiation of the homolateral inguinal, iliacal, paraaortic and renal hilus lymph nodes with a dose of 30 Gy in 3 weeks in 15 fractions. Radiotherapy was performed with 18 MV photons from a linear accelerator (C.G.R.). The mean age was 35.3 years (4.2 S.D.).

In breast cancer patients, both a retro- and a prospective study was performed. The prospective group consisted of 40 patients, the retrospective of 20 women. The eligibility criteria were: female sex, histologically proven adenocarcinoma of the breast, good general condition (Karnofsky index > 80%), no other cancer, no diseases or drugs which may influence immune parameters (e.g. autoimmune diseases, corticosteroids, cycloxygenase inhibitors) and clinical and pathological stage T1NOMO and T2 (< 4 cm) NOMO completely resected.

In the prospective study a blood sample was taken at the time the diagnosis was established with fine-needle biopsy, thus prior to surgery or staging procedures. These values will be referred to as prior to radiotherapy. After staging, tumorectomy and axillary dissection, another blood sample was taken from women fulfilling the eligibility criteria. These results were never significantly different from the first examination. During radiotherapy, and 2–3 months thereafter lymphocyte populations were determined. In the retrospective study, women fulfilling the eligibility criteria and having received radiotherapy 4–6 years ago were studied. The mean age in the prospective group ws 55.2 years (8.9 S.D.) and 62.1 years (10.3) in the retrospective study. The age at initiation of radiotherapy of the latter patients was therefore not significantly different from the patients included in the prospective study.

*Irradiation*

All patients received a dose of 50 Gy in 25 fractions in 5 weeks (2 Gy/day, 5 days/week) to the breast and the homolateral parasternal and supraclavicular lymph node areas. Breast irradiation was performed with a $^{60}$Co machine (Philips). Radiotherapy of the parasternal lymph nodes was carried out for half of the treatment with $^{60}$Co (Philips), and for the other half with 13 MeV electrons from a linear accelerator (C.G.R.). This has been a standard technique in our department for several years. By using a 50:50 mixture of $^{60}$Co photons and 13 MeV electrons, we avoid both excessive oesophageal irradiation associated with $^{60}$Co, and excessive skin doses when only 13 MeV electrons are employed. With both types of irradiation, the parasternal lymph nodes receive the prescribed dose.

*Determination of the lymphocyte subsets*

Blood was taken in heparinised tubes. Leucocytes were separated on Ficoll-Hypaque. Cells were washed, and aliquoted at a concentration of $1 \times 10^6$ cells/ml. Monoclonal antibodies were added, and after an incubation of 20 min at 4°C with intermittent shaking, cells were washed, resuspended and analysed with a FACSTAR flow cytometer (Becton Dickinson). The data were analysed and processed with a Hewlett–Packard computer system. Two-colour fluorescence was obtained by using FITC (fluorescein isothiocyanate) and phycoerythin labelled antibodies.

The following monoclonal antibodies, all purchased from Becton Dickinson, Belgium, were used: leu 4 (CD3—pan T lymphocyte marker), leu 3 (CD4—helper/inducer phenotype), leu 2 (CD8—suppressor/cytotoxic phenotype), 2H4 (CD45R), 4B4 (CDW29), leu 15 (CD11b), leu M3 (CD14—monocyte/macrophage), HLA-DR, leu 11 (CD16—natural killer cell), leu 12 (CD19), leu 16 (CD20), WT31 (αβ chain of the T cell receptor) and TAC (interleukin-2 receptor). The fluorescence levels, which are related to the antigen density on the cell surface, were also recorded. Double stainings with green (FITC-fluorescein isothiocyanate) and red (phycoerythrin) labelled antibodies were done with CD4-45R (identifying "naive" T lymphocytes),CD4-W29 ("memory" T cells), CD8-45R, CD8-W29, CD4-HLA-DR (activated CD4 cells), CD8-HLA-DR (activated CD8 cells), CD4-wT31 (CD4T lymphocytes bearing the αβ chain of the T cell receptor), CD4-TAC (CD4 cells expressing the p55 chain of the interleukin-2 receptor).

*Statistical analysis*

The two-tailed *t*-test was used, with the pretreatment values as a reference. $P$ values $< 0.05$ were considered significant.

## RESULTS

The lymphocyte subsets of 47 healthy volunteers were compared with the values obtained in breast cancer patients before radiotherapy. No differences were observed.

*Irradiation for breast cancer*

Local irradiation resulted in a significant ($P < 0.01$) decrease of the CD4 T cells, already during radiotherapy (Fig. 1). For up to 5 years after radiotherapy, the levels did not change. In contrast, the number of CD8 cells was not changed during or after treatment.

Because of the decrease of CD4 cells, and the unchanged

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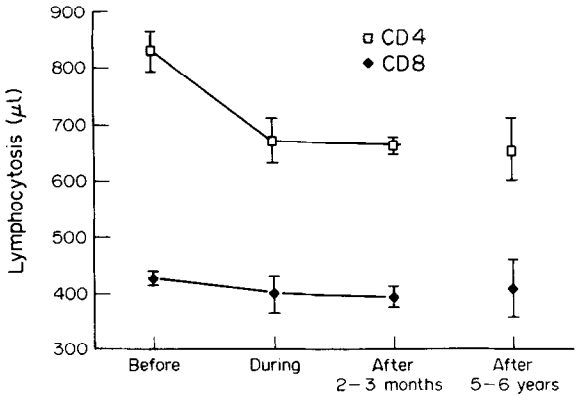


**Fig. 1. Evolution of the absolute number of CD4 and CD8 T lymphocytes after local radiotherapy for breast cancer. The decrease of CD4 cells is significant ($P < 0.01$) as compared to preradiotherapy levels at all time points.**

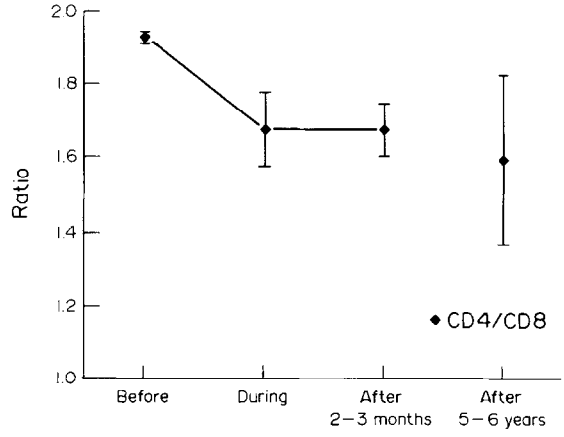


**Fig. 2. CD4/CD8 ratio after local irradiation for breast cancer. The CD4/CD8 ratio decreased significantly ($P < 0.05$) 2–3 months after radiotherapy and remained at that level up to 5–6 years after treatment.**

values of CD8 cells, the CD4/CD8 ratio decreased significantly after irradiation, and remained lower than before treatment up to 5–6 years after radiotherapy (Fig. 2).

As shown in Figs 3 and 4 the decrease of CD4 cells was due to a fall of the CD4-45R ("naive") T cells. The CD4-w29 cells

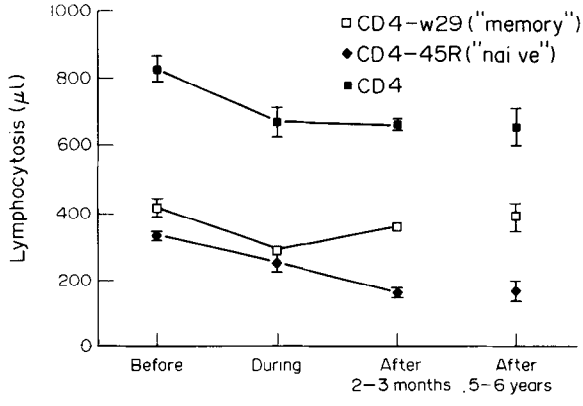


**Fig. 3. Effect of local irradiation for breast cancer on the absolute number of CD4 T lymphocytes, CD4-45R ("naive") and CD4-w29 ("memory") T cells. The long-term decrease of CD4 cells and of CD4-45R cells is significant (respectively $P < 0.01$ and $P < 0.001$).**

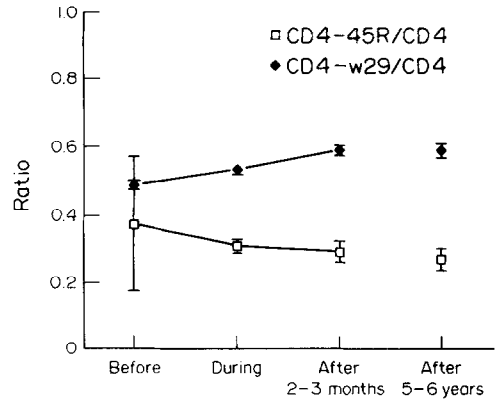


**Fig. 4. Effect of local irradiation for breast cancer on the ratio of CD4-45R/CD4 cells and CD4-w29/CD4 cells. The decrease of the ratio CD4-45R/CD4 cells ("naive" CD4 cells)/total CD4 cells) is significant ($P < 0.05$), as is the increase of the ratio CD4-w29/CD4 ("memory" CD4 cells/total CD4 cells) ($P < 0.001$).**

("memory" T cells) initially decreased during radiotherapy, but recovered within 3 months to their pretreatment levels. It can thus be concluded that human CD8 cells are relatively resistant to local radiotherapy *in vivo*, whereas a subpopulation of CD4 cells (the "naive" cells) is very sensitive to it and can hardly recover.

The number of both HLA-DR positive CD4 and HLA-DR positive CD8 T lymphocytes increased significantly after radiotherapy (Fig. 5).

No significant changes were observed for the CD3 T lymphocytes, the WT-31 + CD3 cells (expressing the α/β chain of the T cell receptor), the NK cells (defined by the CD16 antigen) and the pre-B lymphocytes (CD19 + cells) (Fig. 6). Only the increase of B lymphocytes (CD20 + cells) was significant 5–6 years after treatment ($P < 0.05$).

At no time-point differences in the density of antigen expression were observed (data not shown).

*Irradiation for seminoma of the testis*

To examine the influence of the radiation field (infradiaphragmatic vs. supradiaphragmatic), sex and age, the results of the lymphocyte subpopulations in irradiated breast cancer patients were compared with those obtained in irradiated seminoma testis patients (Table 1) 5–6 years after treatment.

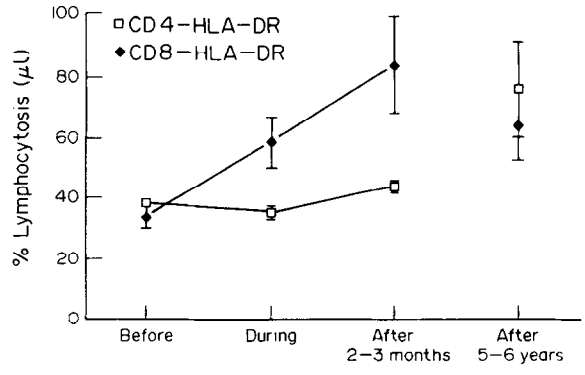


**Fig. 5. Changes of the absolute number of HLA-DR positive CD4 and CD8 cells after local radiotherapy for breast cancer. The increase is significant for the two populations ($P < 0.02$ and $P < 0.01$, respectively).**

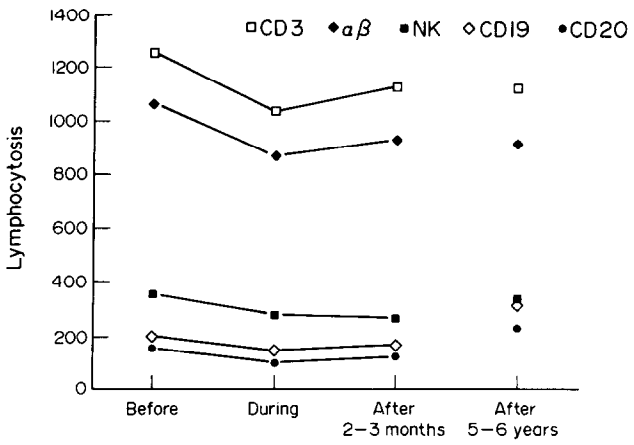


**Fig. 6. Effect of local irradiation for breast cancer on the number of CD3, NK, B, α/β positive CD3 cells, and on B lymphocytes. Only the long-term increase of B cells (CD20) is significant ($P < 0.05$).**

No significant changes were observed. These findings suggest that the long-term immune effects of irradiation observed in cancer patients, were not related to sex, age, anatomical location of the radiation portal, or of the histology of the tumour.

## DISCUSSION

In the present study we investigated prospectively and retrospectively the effects of local radiotherapy on the lymphocyte subsets of breast cancer patients and men with seminoma of the testis. For breast cancer patients, lymphocyte determinations were made using flow cytometry, before, during, 2–3 months and 5–6 years after treatment. All patients had adenocarcinomas of the breast, pathological stage T1-2NOMO. For seminoma testis patients with stage I disease blood was collected 5–6 years after treatment. None of them showed signs of disease activity when blood was taken during the follow-up period, and none had conditions which could have influenced the immune system, like infections, autoimmune disorders or the use of drugs like corticosteroids, cycloxygenase inhibitors or immunosuppressants. No significant differences were observed between the two patient groups, suggesting that the immune alterations induced by radiotherapy were not related to sex, age, anatomical location of the radiation portal, or of the histology of the tumour.

The number of CD4 T lymphocytes decreased significantly during radiotherapy and remained low up to 5–6 years after treatment. The decrease of the CD4 T cells was due to a decrease of the CD4-45R ("naive" T cells) subset. The CD8 cells were not significantly altered by radiotherapy. No changes in antigen density were observed.

The present results confirm previous studies in which it was demonstrated that local radiotherapy causes a long-term decrease of the CD4 T lymphocytes, whereas the CD8 cells soon return to their initial value [1–4, 8, 9]. The degree of lymphopenia observed was less pronounced than in the Swedish studies [2, 3], but is comparable to Yang's results [4]. These differences may be due to the irradiated volume which was larger in the Swedish studies. In the latter series, not only the breast and the parasternal and supraclavicular lymph nodes were treated, but also the homolateral axilla.

The observation that the CD4-45R cells were not capable of recovery after radiotherapy, may be indicative for their intrinsic deficiency to proliferate or for a defect of the thymus of these on average older patients [6] to compensate for the CD4-45R lymphopenia. The fact that CD4-45R positive cells are considered as short-lived, unstimulated lymphocytes emigrating from the thymus [5] and that thymic functions are defective in older patients [6] argues for this hypothesis.

The increase of HLA-DR positive T cells (Fig. 5), is not only related to the relative radioresistance of activated T cells [10], but also implies proliferation of these cells *in vivo* as there was an absolute increase in the numbers of these cells. As HLA-DR antigens appear on activated cells, these cells, which per definition have encountered an antigen, are probably memory cells which will be responsible for the recovery of part of the CD4 population. Studies with sheep treated with the thymidine analogue bromodeoxyuridine (BUdR) showed that the memory subset can proliferate *in vivo* [11].

During the observation period, no significant changes in the number of CD3 cells, CD3-WT31 cells, NK cells or pre-B cells were observed. Only the B cells increased significantly (Fig. 6). A relative increase of B lymphocytes was also described after TLI in humans [12], and in rodents [13]. It is not clear yet whether this is due to an increased intrinsic proliferative capacity, or to altered lymphokine balances involved in B-cell generation [14].

*Table 1. Absolute number (per mm³) of lymphocyte subsets 5–6 years after irradiation for stage I seminoma testis, compared to irradiated stage I–II breast cancer patients*

| Lymphocyte subset | Seminoma testis ($n$ = 10) Mean | S.E. | Breast cancer ($n$ = 20) Mean | S.E. | |
|---|---|---|---|---|---|
| CD3 | 936.2 | 34.0 | 1126.3 | 102.1 | NS |
| CD4 | 566.3 | 9.8 | 655.0 | 55.4 | NS |
| CD4-45R | 227.1 | 4.6 | 168.5 | 33.3 | NS |
| CD4-w29 | 238.7 | 5.0 | 388.2 | 43.0 | NS |
| CD8 | 347.9 | 10.2 | 409.6 | 52.6 | NS |
| CD4-HLA-DR | 52.4 | 0.1 | 76.0 | 15.5 | NS |
| CD8-HLA-DR | 43.6 | 0.1 | 64.0 | 10.9 | NS |
| CD3-WT31 | 790.6 | 25.0 | 919.0 | 42.4 | NS |
| CD16 | 227.1 | 8.6 | 332.2 | 73.0 | NS |

NS = not significant.

A fast recovery of NK activity against the K562 cell line [15–19] or even no change when tested against Chang cells [15, 19] after local radiotherapy have been described. This is thus in accordance with our findings that the NK cells were not significantly changed after local radiotherapy in our patient groups.

Our results may explain why locally irradiated patients do not have a higher incidence of infections [20], despite the observed immune alterations. First, the lymphopenia was not extreme. Second, the NK cells, which are a first line defence cells against viral infected cells [21] and certain bacteria [22], were nearly not affected by local radiotherapy. Third, the memory cells were at normal levels after irradiation, so that it is likely that an adequate immune response can be generated against all the antigens to which an individual was previously exposed.

The above-mentioned arguments regarding defence mechanisms against infections after radiotherapy may also apply to putative antitumour immune responses. If these mechanisms could indeed play a protective role against cancer, it is very likely that an immune memory, which can be built up within a few weeks, did already exist before initiation of local radiotherapy which does not alter the immunological memory. Moreover, because of the high susceptibility of some suppressor cells to irradiation [10] and because of the increase of activated T cells (Fig. 5), it may well be that the net result of local radiotherapy is an augmentation of antitumour immune responses. The observation that local irradiation of an immunogeneic rodent tumour resulted in a dose-dependent infiltration of the tumour with CD4 and CD8 T lymphocytes may support this hypothesis [23].

We showed previously that hindlimb and tail irradiation in mice resulted in an increased CD4/CD8 ratio due to a deficient recovery of the CD8 T cell subset [24, 25]. In humans, the situation was opposite: a decreased CD4/CD8 ratio due to a deficient recovery of the CD4 cells was found (Fig. 1). Both in man [26, 27] and mice [24, 25], the intrinsic radiosensitivity of CD4 and CD8 T lymphocytes is equal.

We propose that both CD4 and CD8 cells are made up by a "thymic" fraction of cells with a rapid turnover and a limited life span, and a "peripheral" fraction of cells which are long-lived and can if necessary—for instance after irradiation—slowly proliferate. If the CD4 cells contain relatively more "thymic" cells, this population could be restored relatively more rapidly when a good thymic function is present. We propose that in mice a relatively important part of the CD4 population is composed of rapidly proliferating recent thymic emigrants, which after irradiation can be rapidly restored by new thymic emigrants. This explains the early recovery of this population after local radiotherapy in normal mice, and the slower recovery of CD4 cells in thymectomised animals. The CD8 population is predominantly composed of T cells which mature outside of the thymus [7]. This explain why there is not much difference in the recovery of CD8 positive cells in thymectomised as compared to non-thymectomised animals. In humans, where the thymic function rapidly declines [6], the recovery of the CD4 cells is not only very slow and deficient, but also limited to the CD4-w29 positive, thus subpopulation which are extrathymic memory cells [5]. Just like in mice, the CD8 population is predominantly composed of extrathymic CD8 cells which are not significantly affected by local radiotherapy.


1. Haas GS, Halperin E, Doseretz D, *et al.* Differential recovery of circulating T cell subsets after nodal irradiation for Hodgkin's disease. *J Immunol* 1984, **132**, 1026–1030.
2. Petrini B, Wasserman J, Blomgren H, Glas U. Changes of blood T cell subsets following radiation therapy for breast cancer. *Cancer Lett* 1983, **10**, 27–31.
3. Rotstein S, Blomgren H, Petrini B, Wasserman J, Baral E. Long term effects on the immune system following local radiation therapy for breast cancer. I. Cellular composition of the peripheral blood lymphocyte population. *Int J Radiat Oncol Biol Phys* 1985, **11**, 921–925.
4. Yang S-J, Rafla S, Youssef E, Selim H, Salloum N, Chuang JY. Changes in T-cell subsets after irradiation therapy. *Radiology* 1988, **168**, 537–540.
5. Sanders ME, Makgoba MW, Shaw S. Human naive and memory T cells: reinterpretation of helper-inducer and suppressor-inducer subsets. *Immunology Today* 1988, **7**, **8**, 195–199.
6. Steinman GG. Changes in the human thymus during aging. *Curr Topics Pathol* 1986, **75**, 43–88.
7. MacDonald R, Blanc C, Lees RK, Sordat B. Abnormal distribution of T cell subsets in athymic mice. *J Immunol* 1986, **136**, 4337.
8. Saper V, Chow D, Engleman ED, *et al.* Clinical and immunological studies of cadaveric renal transplant recipients given total-lymphoid irradiation and maintained on low-dose prednisone. *Transplantation* 1988, **45**, 540–546.
9. Waer M, Vanrenterghem Y, Roels L, *et al.* Immunological and clinical observations in diabetic kidney graft recipients pretreated with total-lymphoid irradiation. *Transplantation* 1987, **43**, 371–379.
10. Anderson RE, Standefer JS, Tokuda S. The structural and functional assessment of cytotoxic injury of the immune system with particular reference to the effects of ionizing irradiation and cyclophosphamide. *Br J Cancer* 1986, **53**, 140–160.
11. Mackay CS, Marston WL, Dudler L. Naive and memory T cells show distinct pathways of lymphocyte recirculation. *J Exp Med* 1990, **171**, 801–817.
12. Fuks Z, Strober S,Bobrove AM, Sasazuki T, McMichael A, Kaplan HS. Long-term effects of radiation on T and B lymphocytes in peripheral blood of patients with Hodgkin's disease. *J Clin Invest* 1976, **58**, 803–814.
13. Slavin S, Strober J, Fuks Z, Kaplan HS. Induction of specific tissue transplantation tolerance using fractionated total lymphoid irradiation in adult mice: long-term survival of allogeneic bone marrow and skin grafts. *J Exp Med* 1977, **146**, 34–48.
14. Bass H, Strober S. Deficits in T helper cells after total lymphoid irradiation (TLI): reduced IL-2 secretion and normal IL-2 receptor expression in the mixed leukocyte reaction (MLR). *Cell Immunol* 1990, **126**, 129–142.
15. Blomgren H, Strender LE, Petrini B, Wasserman J. Changes of the spontaneous cytoxicity of the blood lymphocyte population following local radiation therapy for breast cancer. *Eur J Cancer Clin Oncol* 1982, **18**, 637–743.
16. Blomgren H, Hammarström S, Wasserman J, Petrini B. Prostaglandin sensitivity of the PHA-response of blood lymphocytes following radiation therapy for breast cancer. *Radiother Oncol* 1986, **7**, 141–145.
17. McGinnes K, Florence J, Penny R. The effect of radiotherapy on the natural killer (NK)-cell activity of cancer patients. *J Clin Immunol* 1987, **7**, 210–217.
18. Onsrud M, Thorsby E. Long-term changes in natural killer activity after external pelvic radiotherapy. *Int J Radiat Oncol Biol Phys* 1981, **7**, 609–614.
19. Rotstein S, Blomgren H, Petrini B, Wasserman J, Baral E. Long-term effects on the immune system following local radiation therapy for breast cancer. III. Changes of spontaneous and lectin dependent cellular cytotoxicity. *Anticancer Res* 1986, **6**, 113–118.
20. Rotstein S, Blomgren H, Baral E, *et al.* Incidence of infectious symptoms after radiation therapy for breast cancer. Long-term effects. *Acta Oncol* 1987, **26**, 197–201.
21. Whiteside TL, Herberman RB. Short analytical review. The role of natural killer cells in human disease. *Clin Immunol Immunopathol* 1989, **53**, 1–23.
22. Garcia-Penarrubia P, Koster FT, Kelley RO, McDowell RD, Bankhurst AD. Antibacterial activity of human natural killer cells. *J Exp Med* 1989, **169**, 99–113.
23. Hirota S, Ogawa Y, Seguchi H. Histological analysis of lymphocyte subsets infiltrated into mouse tumor tissue exposed to local irradiation. *Oncology* 1987, **44**, 312–318.
24. De Ruysscher D, Waer M, Vandeputte M, van der Schueren E. Immunologic changes after loco-regional radiotherapy and fraction-

ated total body irradiation (TBI) mice. *Int J Radiat Oncol Biol Phys* 1989, **17**, 1237–1245.
25. De Ruysscher D, Waer M, Vandeputte M, van der Schueren E. Mechanisms involved in the differential recovery of CD4 and CD8 T-lymphocytes after local irradiation in mice. *Radiother Oncol* 1990, **18**, 299–305.
26. Dutreix J, Girinski T, Casset JM, *et al.* Blood cell kinetics and total body irradiation. *Radiother Oncol* 1987, **9**, 119–129.
27. Manori I, Kushilevsky A, Segal S, Weinstein Y. Radiosensitivity of isolated subsets of human lymphocytes ($E^+$,$OKT4^+$,$OKT8^+$): protective role of monocytes and monokines. *Clin Exp Immunol* 1984, **58**, 453–461.

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# Feature Articles

# Photodynamic Therapy

**Thomas J. Dougherty and Stuart L. Marcus**

**Photodynamic therapy (PDT) has been developed over the past decade into a useful treatment for several types of solid cancers in man. This unique therapy requires a photosensitiser accumulated in tumours and local activation by visible light generally delivered from lasers and delivered to the patient through various types of fibers and endoscopes. PDT appears to be most effective in treating certain superficial, difficult to treat cancers such as carcinoma *in situ* of the urinary bladder (here complete control is the intent), but also is effectively used in bulkier tumours obstructing bronchi or the oesophagus where palliation can be achieved. The primary mechanism of action is the *in situ* generation of an active form of molecular oxygen (singlet oxygen) which causes the rapid, local onset of vascular stasis and eventual vascular haemorrhage and tumour wall destruction. This process appears to be mediated through various cytokines such as prostaglandin, lymphokines and thromboxanes. The ultimate clinical value of PDT will be seen over the next few years following health agency approval worldwide.**
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## INTRODUCTION

WHILE MOST cancer deaths are a result of advanced metastatic disease, a considerable number of patients succumb due to inability to control local tumours (e.g. glioblastoma multiforme, certain intraperitoneal cancers). In some cases, potentially curable early stage cancers cannot be effectively treated by standard therapy because of mitigating clinical situations (e.g. early stage lung cancers or refractory carcinoma *in situ* of the urinary bladder in elderly patients with medical conditions precluding surgery). In still other situations, the therapeutic intent is palliation with as little adverse reaction as possible. Photodynamic therapy (PDT), a relatively selective, local treatment, has been examined in all these situations in order to define its role in cancer treatment, both for palliation and complete local tumour eradication. Several ongoing Phase III clinical trials for health agency approval in the USA, Canada, Europe and Japan are described below, along with clinical experience with PDT from certain earlier Phase I/II trials.

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## METHODOLOGY AND MECHANISMS

PDT requires a photosensitiser, localised with some degree of selectivity in the solid tumour and the means for its local activation. The latter is achieved generally by visible light derived from a laser and directed to the site by various fibre optics. The cytotoxic process which occurs with the porphyrin currently in clinical trials (Photofrin, porfimer sodium) as well as with most experimental new photosensitisers, is known as the photodynamic process, a type II photochemical reaction:

$$PS + h\nu \rightarrow PS^*(1)$$
$$PS^*(1) \rightarrow PS^*(3)$$
$$PS^*(3) + O_2 \rightarrow PS + O_2^*$$
$$^*O_2 + T \rightarrow \text{cytotoxicity}$$

where PS = photosensitiser, PS*(1) = excited singlet state of PS, PS*(3) = excited triplet state of PS, $h\nu$ = light quantum, $O_2^*$ = excited singlet state of oxygen and T = cellular target.

In practice, the patient receives an intravenous injection of Photofrin (1–2 mg/kg) and 24–72 h later is treated with 630 nm light from a dye laser directed through single quartz fibers with a variety of speciality ends. Photofrin has no apparent pharmacological effect in the absence of light activation, although the spleen and bone marrow in mice demonstrate increased cellularity [1]. However, its retention in skin requires