Pergamon

European Journal of Cancer Vol. 31A, No. 10, pp. 1586–1591, 1995
Elsevier Science Ltd
Printed in Great Britain.
0959–8049/95 $29.00+0.00

0959-8049(95)00302-9

Review

Haemopoietic Stem Cell Inhibition: Potential for Dose Intensification

E. Marshall

INTRODUCTION

THE CONCEPT of a dose–response curve in experimental tumour models has been fundamental to the development of chemotherapeutic protocols [1, 2]. Currently, there is considerable interest in dose intensification as a means of improving response rates and ultimately survival. Retrospective studies by Hryniuk in breast carcinoma [3, 4] showed disease outcome is significantly correlated with the relative received dose intensity, and this has been rapidly confirmed in other solid tumours [5–8]. Retrospective analyses, however, are open to criticism and the ability to test the hypothesis has been limited by cytotoxic-induced neutropenia and thrombocytopenia. The advent of haemopoietic growth factors [9, 10] has enabled a greater and more frequent dose delivery with corresponding improvement in response rates, but the modest increase in dose intensity (typically <2-fold) has resulted in minimal benefit in terms of overall survival [11]. In addition, the small gain has been compounded by enhanced toxicity on other tissues, most notably epithelium and megakaryocytes, with significant patient morbidity and mortality. Experimental animal models have also shown that the recovery of blood differential counts bears no relationship to the viability and kinetics of the progenitor population [12], and that repeated treatment with myelosuppressive agents and growth factors may result in incremental damage to the stem cell pool [13].

The ability to protect normal host tissues, at the expense of malignant cells, offers an alternative and more attractive approach for dose intensification, and has been achieved by a number of different experimental approaches. The aminothiols, most notably WR 2721 (Amifostine, Ethyol), are currently in phase III clinical trials and may offer selective protective effects against myelotoxicity, neurotoxicity and nephrotoxicity, possibly by free radical scavenging [14, 15]. Gene therapy techniques remain at the embryonic stage, but there is already exciting evidence to show myeloprotection following retroviral transfection of alkyltransferase, a DNA repair enzyme [16]. This review will focus on a new class of agents, the stem cell inhibitors [17–19], which offer a novel approach for protecting normal host tissues by manipulating cell cycle regulation. They may, therefore, allow delivery of a greater therapeutic dose intensity in the short term, together with maintenance of a viable stem cell pool in the long term.

Correspondence to E. Marshall at the CRC Department of Medical Oncology, Christie CRC Research Centre, Christie Hospital NHS Trust, Wilmslow Road, Manchester M20 9BX, U.K.
Received 16 Mar. 1995; accepted 10 May 1995.

HAEMOPOIETIC STRUCTURE AND CONTROL MECHANISMS

Haemopoiesis represents an ideal model for studying the control mechanisms and interactions of a continuously regenerating system which can adapt rapidly to changing requirements. Within this system, a small population of self-renewing and pluripotent stem cells can generate an increasing number of lineage-restricted progenitors which themselves differentiate to morphological recognisable effector cells. The precise mechanisms underlying lineage commitment remain obscure, but the development of *in vitro* clonogenic assays has highlighted the importance of a large number of glycoproteins—haemopoietic growth factors—which play a central role in regulating proliferation and differentiation [9, 10]. Investigation and characterisation of the human stem cell population has proved extremely difficult because of the inability to assay these cells directly, and consequently much of the work is based on a murine stem cell assay that quantitates a heterogeneous population termed spleen colony-forming units (CFU-S) [20].

Despite the advances in growth factor development, the recognition that stem cells are normally in a resting, G_0 state [21, 22], and the fact that positive feedback alone is an untenable concept, led to the recognition of several unrelated agents which feedback to maintain stem cell quiescence and perhaps, ultimately the balance between self-renewal and proliferation. Several such inhibitors have now been more clearly defined and include macrophage inflammatory protein (MIP)-1α [23], AcSDKP (acetyl-N-Ser–Asp–Lys–Pro) [24], pEEDCK (pyroGlu–Glu–Asp–Cys–Lys) [25] and transforming growth factor (TGF)-β [26], although their precise physiological roles and interactions await elucidation.

The ability to maintain stem cells in a quiescent state offers distinct clinical advantages with respect to protection from cycle-specific cytotoxic agents as exemplified by the antimetabolites, cytosine arabinoside and 5-fluorouracil. Under steady state conditions, the rapidly dividing progenitor population receives the brunt of cytotoxic-induced damage, and the reduced output of functional cells results in the well-recognised myelosuppression. Subsequently, the normally quiescent stem cell population

is recruited into DNA synthesis to replenish the depleted progenitor compartment [27–30].

The normalisation of neutrophils and platelets to predefined levels has historically been used as an index of bone marrow recovery. However, this is not supported by animal data which reveal a disparity between blood count recovery and the return to stem cell quiescence [12]. Repeated administration of chemotherapy at a time when a significant number of stem cells remain in DNA synthesis will reduce the stem cell pool, both from direct cytotoxicity and loss through differentiation [28, 31]. The consequences of repeated insult on the stem cell population, following multicyclic chemotherapy, are only too familiar to the oncologist. The resulting delay in neutrophil and platelet recovery inevitably necessitates a reduction in dose intensity in a significant proportion of patients, and ultimately may have serious adverse effects on response and survival [32, 33].

The addition of a stem cell-specific inhibitor around the time of chemotherapy has the potential to prevent stem cell recruitment by overriding the proliferative drive, and thus maintain sufficient numbers to allow an enhanced recovery of the haemopoietic tissue from a higher platform.

This model is almost certainly an oversimplification, but is justified by experimental evidence [25, 34, 35]. The theory is more difficult to apply to the non-cycle-acting chemotherapeutic agents (alkylating species, anthracyclines) or radiation. In this setting, cell death occurs largely independently of cycle status and would suggest that proliferation inhibition has little role to play. This is currently the subject of intense study but several observations are pertinent. Many cytotoxics, including anthracyclines and alkylating agents, induce a greater cell kill in cell lines which are synchronised in S phase [36, 37], and this is borne out by clinical observation. On a more esoteric note, the mode of toxicity induced by alkylating species may also be open to manipulation. Alkylating agents represent a major class of chemotherapeutic drugs with activity in a wide spectrum of malignancies. The formation of DNA adducts and the presence of unrepaired adducts at the time of DNA replication represents the principal cytotoxic event of these compounds [38, 39]. A proportion of cells, presumably including haemopoietic stem cells, also sustain sublethal mutational damage [38]. Propagation of this mutational damage can lead to tumour initiation [39], and it is likely, although less well defined, that similar mechanisms exist to account for delayed bone marrow failure. Specific DNA repair enzymes have been identified [40], but the time available for completion of this process is likely to be a critical factor.

One can hypothesise that the loss of feedback inhibition, resulting from cytotoxic treatment, will reduce the degree of DNA repair given the proliferation pressure on the stem cell and reduced cell cycle time. In this scenario, "inhibitors" may not protect in the same sense, but may allow recovery from more viable populations by providing more time for housekeeping genes to complete all necessary repair steps.

MACROPHAGE INFLAMMATORY PROTEIN (MIP)-1α

MIP-1α is an 8-kDa polypeptide that was isolated in doublet form with MIP-1β as MIP-1 [41]. The gene for the human homologue, LD78, has been sequenced and cloned and is located on the long arm of chromosome 17 at q11-q21 [42]. On the basis of structural homology, MIP-1α is classified as a member of the chemokine or scy (small cytokine) family—other close relatives including platelet factor 4 (PF4), interleukin-8 (IL-8) and rantes [43]. All members are basic heparin-binding polypeptides and are intimately involved in inflammation and chemotaxis [43]. A limited number, including MIP-1α, MIP-2α, PF4, IL-8 and monocyte chemotactic and activating factor (MCAF), appear to share proliferation modulating effects on immature haemopoietic colony-forming cells (CFCs) [44, 45]. In physiological buffers, MIP-1α readily forms large multimeric complexes due to non-covalent self-aggregation [46]. The biological role of this phenomenon is unclear, but it poses a number of problems with respect to drug development and interpretation of dose–response assays. Current phase I trials utilise a non-polymerising variant of MIP-1α, produced by site-directed mutagenesis, which retains wildtype activity in both receptor binding assays and *in vivo* animal models (data not shown).

In 1976, Lord and associates isolated a bone marrow extract which could inhibit haemopoietic stem cells [47], but it was not until 1990 that the active moiety—MIP-1α—was identified and characterised [23]. Subsequently, a large wealth of knowledge has been accrued on the physiological role of MIP-1α as a central player in the feedback control mechanisms governing haemopoiesis [48].

The stem cell compartment is recognised to be a heterogeneous population with an age stratification and variable self-renewal. MIP-1α is most specific for the maturing, older subset of multipotent stem cells with consistent inhibitory effects on CFU-S and the *in vitro* equivalent, CFU-A [49, 50]. Inhibition may not extend to the most primitive pluripotent cells that possess marrow repopulating ability [51, 52]. The increasingly lineage-restricted progenitor cells are also resistant to the inhibitory effects of MIP-1α [23, 51, 53]. Paradoxically, a number of investigators has reported growth stimulation due to MIP-1α in the presence of specific growth factors [49, 53–55], but the results have been conflicting and are largely determined by the combination of cytokines present. MIP-1α does not have colony-stimulating activity *per se*.

The clinical potential of MIP-1α was highlighted simultaneously by Lord and associates [34] and Dunlop and associates in 1992 [50], using a murine model with hydroxyurea (HU) and cytosine arabinoside, respectively. As discussed earlier, initial dosing with an S-phase killing agent produces a relatively small stem cell kill, but triggers recruitment into DNA synthesis. Following HU treatment, CFU-S are semisynchronised in S-phase approximately 7 h later [56]. Further cytotoxic administration at this time point results in a much greater reduction in CFU-S numbers. The addition of MIP-1α before the second cytotoxic administration prevents recruitment, with optimal doses producing complete protection of the stem cell compartment [34] and a shortened neutropenic period [50]. Of further significance, the suboptimal dose schedules not only enhanced recovery of CFU-S but appeared to improve self renewal—a finding that has since been substantiated in both animal models and long-term bone marrow culture (LTBMC) [57, 58].

The observation that stem cells, released from a proliferation block, have an enhanced self-renewal performance offers exciting prospects and possible therapeutic advantages beyond the boundaries of S-phase cytotoxics. In clinical practice, multicyclic scheduling is considered the optimum delivery of chemotherapy, and despite advances in transplantation techniques, is likely to remain the major approach to both palliation and cure. The incremental bone marrow damage which follows repeated cytotoxic insults has been explained by an aging of the pluripotent stem population with reduction in their self-renewal capacity, or alternatively impairment of their proliferative ability resulting from stem cell mutations [59–61]. The two are not necessarily

mutually exclusive, and either scenario may benefit from the self-renewing enhancement properties of MIP-1α.

The ability to modify recovery curves following repeated multicyclic therapy has been confirmed by Lord and associates using a murine model of repeated sublethal radiation [62]. A control group receiving 2-weekly cycles of 4.5 Gy gamma rays sustained incremental damage at the CFU-S level. In a second cohort receiving MIP-1α, a consistent improvement in CFU-S recovery was seen and was accentuated with each cycle. This advantage occurs despite an apparent lack of any direct radioprotective effect as judged by similar CFU-S nadir numbers. The enchancement of cell numbers is maintained through the hierarchy of stem cells including the most primitive marrow repopulating cells (Dr B.I. Lord, Paterson Institute, Manchester, U.K.).

The cellular mechanism underlying the dual actions of MIP-1α have yet to be elucidated. The inhibitory effect is recognised to be direct with experimental evidence from single-sorted $CD34^+$ cells and highly purified subpopulations [44]. Although many questions remain unanswered, the current experimental evidence provides a compelling argument for the use of MIP-1α with combination chemotherapy, irrespective of the mode of cytotoxicity.

OTHER INHIBITORS

Numerous unrelated molecules have antiproliferative effects on the haemopoietic system, but these are largely non-specific and indirect. However, there are several agents which have undergone extensive preclinical study and appear to be further candidates for physiological regulators of stem cell proliferation. Two of these, AcSDKP [24] and pEEDCK [25], are oligopeptides with high specificity for haemopoietic tissues, the third, TGF-β [26], is a glycoprotein that is both ubiquitous and pleiotropic in nature.

The pentapeptide (pEEDCK) was first isolated in 1982 [63] as a product of mature leucocytes, and at the present time remains in the preclinical phase. Its cellular specificity is similar to MIP-1α, but may extend to the more mature progenitors including granulocyte–macrophage, colony-forming cells (GM-CFC) [64]. The peptide increases survival in mice following lethal doses of cytosine [65], and in a more clinically relevant model has been shown to reduce the duration of neutropenia following cytotoxic therapy [66]. The pEEDCK monomer is easily oxidised to a dimer which paradoxically has colony-stimulating activity [66, 67]. Given the ease of monomeric oxidation, one could anticipate considerable difficulty in reproducing biological effects. Combination experiments indicate that the sequential use of monomer and dimer completely abrogates chemotherapy-induced neutropenia [66, 67].

In 1977, Frindel and associates isolated a stem cell inhibitor in fetal calf bone marrow [68]. The inhibitor has subsequently been identified as the tetrapeptide, AcSDKP, and is the first member of this class of agent to enter clinical trial (seraspenide) [69]. AcSDKP is derived from thymosine β-4 [70], and is synthesised endogenously in LTBMC [71]. Unlike MIP-1α, the inhibitory effect is thought to be indirect [72]. Experimental models have confirmed protection against S-phase-specific drugs by preventing recruitment of stem cells into cell cycle [73, 74], in addition to survival benefits in cyclophosphamide-treated mice [75], and *in vitro* protection against 3′-azido-3′deoxythymidine (AZT) [76].

The first phase I/II study with seraspenide was undertaken in 1991 in cancer patients receiving two consecutive cycles of monochemotherapy (cytosine or ifosfamide) [69]. Tolerance was excellent and a protective effect was demonstrated when comparing both the leucocyte area under the curve (AUC) and the duration and depth of neutropenia. The study highlighted a number of problems with the introduction of these agents—that of trial design and endpoint. Given the excellent tolerance of these agents and isolated effects on a hidden population, phase I dose-finding studies may not be informative. Furthermore, the protective effect as seen in crossover studies may be diluted by the unprotected cycle. A similar ongoing trial in Glasgow, U.K., utilising single-agent doxorubicin, is also underway [77], but is once again hindered by a crossover design which may hide any small but significant benefit that would occur following the ideal of protecting every cycle.

TGF-β has wide-reaching effects which are determined by the target cell and local environment [78]. In addition to haemopoietic stem cell inhibition [26, 79], TFG-β also plays a leading role in cellular differentiation, wound healing and extracellular matrix formation [80]. Although it has been shown to prevent stem cell exhaustion in a cytotoxic model using stem cell factor (SCF) [81], its toxicity and pleiotropic nature are likely to prove major obstacles in clinical development. A significant advantage arises from the extension of its inhibitory effects to other clinically relevant tissues. The ability to reduce oral mucositis [82] will have considerable clinical impact, and may prove far more rewarding than haemopoietic protection given the escalating use of peripheral blood stem cells for transplantation purposes.

EFFECTS ON NON-HAEMOPOIETIC TISSUES

The increasing application of high-dose therapy with growth factors in cancer patients has produced a greater awareness of dose-limiting toxicities beyond the recognition of haemopoietic damage. Other rapidly dividing tissues, particularly oral and gut mucosa, have a similar stem cell structure, and are restrained by corresponding but less well-defined, feedback mechanisms [83]. TFG-β represents one of the most potent inhibitors of epithelial tissues, and experimental models show comparable protection by virtue of cycle inhibition [82]. Although less well documented, MIP-1α also has regulatory effects on other self-renewing tissues, e.g. epidermis, suggesting a possible role as a pan-stem cell regulator [84, 85]. There are no reports on epithelial tissue regulation by either AcSDKP or pEEDCK, but the former is known to regulate proliferation in adult rat hepatocytes [86].

The application of stem cell inhibitors to the field of oncology is clearly dependent on their specificity for normal host tissues. Extensive experimental evidence is lacking, but preliminary work, utilising both haemopoietic and solid tumour cell lines, suggests this may be the case [66, 75, 87]. This has been most elegantly shown using an *in vitro* model of chronic myeloid leukaemia (CML) grown in LTBMC [86, 88]. In LTBMC, an adherent stromal layer can maintain haemopoiesis for at least 12 weeks in the absence of exogenous growth factors [89]. The primitive haemopoietic progenitors located in the adherent layer undergo cyclical changes in proliferation dictated by a weekly replacement of medium ("feed") and the proliferation inhibitory actions of TGF-β, MIP-1α and AcSDKP [87, 88, 90]. By contrast, CML progenitors cultured in identical conditions remain in a highly proliferative state, due at least in part to their resistance to MIP-1α [88]. This loss of feedback inhibition may be an intrinsic quality of the malignant phenotype, but it is unlikely to be a universal finding, as exemplified by the growth modulating effects of TFG-β on several tumour cell lines

[78, 88], and the recently reported inhibitory effects of MIP-1α on progenitors from patients with acute myeloid leukaemia [91]. Clearly caution is required until the precise role of physiological inhibitors in carcinogenesis is delineated further.

CONCLUSION

The current practice of bone marrow stimulation with growth factors exploits the enormous generating potential of the haemopoietic system, and has allowed a modest increase in dose intensity. The major drawback with this approach results from the occult damage to the increasingly sensitised progenitor and stem cell compartments which, at least in theoretical terms, may predispose to stem cell exhaustion. In reality, the intensified approach is associated with significant acute damage to the marrow and other rapidly regenerating tissues, and has considerable impact on the long-term recovery and ability to withstand subsequent chemical or infectious insult. The ability to protect stem cells (haemopoietic and non-haemopoietic) offers an attractive approach for testing the hypothesis for dose intensification without over-stressing the system beyond physiological limits. Furthermore, inhibitors and stimulators are not mutually exclusive, and combination therapy may be the ideal goal for the future.

The major challenge now is the development of appropriate and informative clinical trials which may differ in many respects from the more conventional phase I and II studies evaluating the currently available cytokines. With the exception of TGF-β, a maximum tolerated dose (MTD) may not be definable as the inhibitors have been devoid of toxicity in animal studies. This has been borne out in ongoing clinical trials with AcSDKP [69, 77] and MIP-1α (data not shown). In addition, the nature of the stem cell negates the use of conventional dose-finding phase I studies because of the lack of any meaningful biological endpoint. Hence, these agents are only evaluable following stem cell recruitment which necessitates combined chemotherapy/inhibitor protocols, preferably carried out in large, multicentre, randomised trials. The alternative strategy of a crossover design has been employed by the French and Scottish groups with seraspenide (AcSDKP) and does allow smaller cohorts of patients to be analysed, but is handicapped by the carry-over effect of protected cycles and the reduction in optimal benefit, gained by protecting all cycles.

Currently available research shows stem cell inhibition to be a realistic mode of protection, thus allowing optimum delivery of chemotherapy in both the conventional and multicyclic high-dose therapy arena. Despite 20 years of basic scientific investigation, the recent recognition of peripheral blood stem cell mobilisation [92] and *in vitro* stem cell expansion [58] with MIP-1α, highlights the urgency for further study before oncologists can fully exploit the clinical potential of these novel agents.

1. Skipper HE. Criteria associated with destruction of leukaemia and solid tumour cells in animals. *Cancer Res* 1967, **27**, 2636–2645.
2. Frei E III, Canellos GP. Dose: a critical factor in cancer chemotherapy: *Am J Med* 1980, **69**, 585–594.
3. Hryniuk WM. The importance of dose intensity in the outcome of chemotherapy. In De Vita VT, Hellman S, Rosenberg SA, eds. *Important Advances in Oncology*. Philadelphia, JB Lippincott, 1988, 121–142.
4. Hryniuk W, Bush H. The importance of dose intensity in chemotherapy of breast cancer. *J Clin Oncol* 1984, **2**, 1281–1288.
5. Murry N. The importance of dose and dose intensity in lung cancer chemotherapy. *Semin Oncol* 1987, **14** (suppl. 4), 20–28.
6. Hryniuk W, Levine MN. Analysis of dose intensity for adjuvant chemotherapy trials in ovarian cancer. *J Clin Oncol* 1986, **4**, 1162–1170.
7. Magrath IT, Steinberg SM, Adde MA, Haddy TB. Dose rate: an important prognostic determinant in non-Hodgkin's lymphomas in young patients. *Blood* 1989, **74** (suppl. 1), A80.
8. Carde P, Mackintosh R, Rosenberg SA. A dose and time response analysis of the treatment of Hodgkin's disease. *J Clin Oncol* 1983, **1**, 146–153.
9. Metcalf D. Peptide regulatory factors, haemopoietic growth factors. *Lancet* 1989, **1**, 825–827, 885–887.
10. Groopman JE, Molina JM, Scadden DT. Haemopoietic growth factors biology and clinical applications. *N Engl J Med* 1989, **321**, 1449–1459.
11. Le Chevalier T. Dose optimisation and intensification of cytotoxics in solid tumours supported by haemopoietic growth factors. *Eur J Cancer* 1994, **30A**, 410–412.
12. Lord BI. The recovery of bone marrow following cytotoxic treatment. *Med Sci Res* 1988, **16**, 37–38.
13. Hornung RL, Longo DL. Haematopoietic stem cell depletion by restorative growth factor regimens during repeated high dose cyclophosphamide therapy. *Blood* 1992, **80**, 77–83.
14. Glover D, Glick J, Weiler C, *et al.* Phase I trials of WR-2721 and cisplatinum. *Int J Radiat Oncol Biol Phys* 1984, **10**, 1781–1784.
15. Glick J, Kemp G, Rose P, *et al.* A randomised trial of cyclophosphamide and cisplatin ± WR-2721 in the treatment of advanced epithelial ovarian cancer. *Proc Am Soc Clin Oncol* 1992, **11**, A123.
16. Allay JA, Dumenco LL, Gerson SL. Induction of nitrosourea resistance by retroviral transduction of the human alkyltransferase gene into haematopoietic cells. *Exp Haematol* 1994, **22**, A698.
17. Axelrad AA. Some haemopoietic negative regulators. *Exp Haematol* 1990, **18**, 143–150.
18. Wright EG, Pragnell IB. Stem cell proliferation inhibitors. *Balliere's Clin Haematol* 1992, **5**, 723–739.
19. Lord BI. Feedback regulators in normal and tumour tissues. *J Cell Sci* 1988, **10** (suppl.), 231–242.
20. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 1961, **14**, 213–222.
21. Lajtha LG, Pozzi LV, Schofield R, Fox M. Kinetics of properties of haemopoietic stem cells. *Cell Tissue Kinet* 1969, **2**, 39–49.
22. Becker AJ, McCulloch EA, Siminovitch L, Till JE. The effects of differing demands for blood cell production on DNA synthesis by haemopoietic colony forming cells of mice. *Blood* 1965, **26**, 296–308.
23. Graham GL, Wright EG, Herwick R, *et al.* Identification and characterisation of an inhibitor of haemopoietic stem cell proliferation. *Nature* 1990, **344**, 442–444.
24. Lenfant M, Wdzieczak-Bakala J, Guittet E, Prome JC, Sotty D, Frindel E. Inhibitor of haematopoietic pluripotent stem cell proliferation: purification and determination of its structure. *Proc Natl Acad Sci USA* 1989, **36**, 779–782.
25. Paukovits WR, Guigon M, Binder KA, Hengl A, Laerum OD, Schulte-Herman R. Prevention of haematotoxic side effects of cytotoxic drugs in mice by a synthetic haemoregulatory peptide. *Cancer Res* 1990, **50**, 328–332.
26. Keller JR, McNeice IK, Sill KT, *et al.* Transforming growth factor Beta directly regulates primitive murine haematopoietic cell proliferation. *Blood* 1990, **3**, 596–602.
27. Morse SE, Pencricca WJ, Stohlman F Jnr. The effect of hydroxyurea on differentiated marrow erythroid precursors. *Proc Soc Exp Biol Med* 1969, **130**, 986–989.
28. Vassort F, Frindel E, Tubiana M. Effects of hydroxyurea on the kinetics of colony forming units of bone marrow in the mouse. *Cell Tissue Kinet* 1971, **4**, 423–431.
29. Harrison DE, Lerner CP. Most primitive haemopoietic stem cells are stimulated to cycle rapidly after treatment with 5-fluorouracil. *Blood* 1991, **81**, 1237–1240.
30. Lord BI, Woolford LB. Proliferation of spleen colony forming units ($CFU\text{-}S_8$, $CFU\text{-}S_{13}$) and cells with marrow repopulating ability. *Stem Cells* 1993, **11**, 212–217.
31. Vassort F, Winterholer M, Frindel E, Tubiana M. Kinetic parameters of bone marrow stem cells using *in vivo* suicide by tritiated thymidine or by hydroxyurea. *Blood* 1973, **41**, 789–796.
32. Skipper H. Data and analyses having to do with the influence of dose intensity and duration of treatment (single drugs and combinations) on lethal toxicity and therapeutic response of experimental neoplasms. *Southern Res Inst Booklet*, 1986, **13**.
33. Hryniuk WM. More is better. *J Clin Oncol* 1988, **6**, 1365–1367.

34. Lord BI, Dexter TM, Clements JM, Hunter MA, Gearing AJH. Macrophage inflammatory protein protects multipotent haematopoietic cells from the cytotoxic effects of hydroxyurea *in vivo*. *Blood* 1992, **79**, 2605–2609.
35. Guigon M, Mary ZY, Enouf J, Frindel E. Protection of mice against lethal doses of 1-β-D-arabinofuranosylcytosine by pluripotent stem cell inhibitors. *Cancer Res* 1982, **42**, 638–641.
36. Kim SH, Kim JH. Lethal effect of adriamycin on the division cycle of HeLa cells. *Cancer Res* 1972, **32**, 323–325.
37. Dewys WD, Goldin A, Mantel N. Haematopoietic recovery after large doses of cyclophosphamide: correlation of proliferative state with sensitivity. *Cancer Res* 1970, **30**, 1692–1697.
38. Singer B. 0-alkyl pyrimidines in mutagenesis and carcinogenesis: occurrence and significance. *Cancer Res* 1986, **46**, 4879–4885.
39. Lawley PD. Carcinogenesis by alkylating species. In Searle CD, ed. *Chemical Carcinogens*. Washington DC, American Chemical Society, 1976, (ACS Symp Series 173), 83–244.
40. Sancar A, Sancar GB. DNA repair enzymes. *Annu Rev Biochem* 1988, 57, 29–67.
41. Davetelis G, Tekamp-Olson P, Wolpe SD, *et al.* Cloning and characterisation of cDNA for murine macrophage inflammatory protein (MIP), a novel monokine with inflammatory and chemokinetic properties. *J Exp Med* 1988, **167**, 1939–1944.
42. Irving SG, Zipfel PF, Balle J, *et al.* Two inflammatory mediator cytokine genes are closely linked and variably amplified on chromosome 17q. *Nucl Acids Res* 1990, **18**, 3261–3270.
43. Wolpe SD, Cerami A. Macrophage inflammatory proteins 1 and 2: members of a novel superfamily of cytokines. *FASEB J* 1989, **3**, 2565–2573.
44. Lu L, Xiao M, Grigsby S, *et al.* Comparative effects of suppressive cytokines on isolated single $CD34^{++}$ stem/progenitor cells from human bone and umbilical cord blood plated with and without serum. *Exp Haematol* 1993, **21**, 1442–1446.
45. Broxmeyer HE, Sherry B, Cooper S, *et al.* Comparative analysis of the human macrophage inflammatory protein family of cytokines (chemokines) on proliferation of human myeloid progenitor cells: interacting effects involving suppression, synergistic suppression and blocking of suppression. *J Immunol* 1993, **150**, 3448–3458.
46. Wolpe SD, Davatelis G, Sherry B, *et al.* Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. *J Exp Med* 1988, **167**, 570–581.
47. Lord BI, Mori KJ, Wright EG, Lajtha LG. An inhibitor of stem cell proliferation in normal bone marrow. *Br J Haematol* 1976, **34**, 441–445.
48. Lord BI, Heyworth CM, Woolford LB. Macrophage inflammatory protein. Its characteristics, biological properties and role in the regulation of haemopoiesis. *Int J Haematol* 1993, **57**, 197–206.
49. Clements JM, Craig S, Gearing AJH, *et al.* Biological and structural properties of MIP-1α expressed in yeast. *Cytokine* 1992, **4**, 76–82.
50. Dunlop DJ, Wright EG, Lorimore S, *et al.* Demonstration of stem cell inhibition and myeloprotective effects of SCI/rh MIP-alpha *in vivo*. *Blood* 1992, **79**, 2221–2225.
51. Quesniaux VFJ, Graham GJ, Pragnell IB, *et al.* Use of 5-fluorouracil to analyse the effect of macrophage inflammatory protein-1alpha on long-term reconstituting stem cells *in vivo*. *Blood* 1993, **81**, 1497–1504.
52. Scheider JG, Moore MAS. TGF-beta, but not macrophage inflammatory protein-1alpha (MIP-1alpha) abrogates IL-1 and ckit ligand (KL) induced proliferation of murine high proliferative potential (HPP) colonies. *Blood* 1991, **78**, A259.
53. Broxmeyer HE, Sherry B, Lu L, *et al.* Myelopoietic enhancing effects of murine macrophage inflammatory proteins 1 and 2 on colony formation *in vitro* by murine and human bone marrow granulocyte/macrophage progenitor cells. *J Exp Med* 1989, **170**, 1583–1594.
54. Keller JR, Bartelmez SH, Sitnicka E, *et al.* Distinct and overlapping direct effects of macrophage inflammatory protein-1alpha and transforming growth factor beta on haematopoietic progenitor/stem cell growth. *Blood* 1994, **84**, 2175–2181.
55. Broxmeyer HE, Sherry B, Lu L, *et al.* Enhancing and suppressing effects of recombinant murine macrophage inflammatory proteins on colony formation *in vitro* by bone marrow myeloid progenitor cells. *Blood* 1990, **76**, 1110–1116.
56. Hodgson GS, Blacket NM. *In vivo* synchronisation of haemopoietic stem cells with hydroxyurea. *Exp Haematol* 1977, 5, 423–426.
57. Lord BI. MIP-1alpha increases the self-renewal of the haemopoietic spleen colony forming cells following hydroxyurea treatment. *Growth Factors* 1995, in press.
58. Verfaille CM, Catanzarro P, Li W. Macrophage inflammatory protein-1alpha interleukin 3 and diffusible marrow stromal factors maintain human haematopoietic stem cells for at least eight weeks *in vitro*. *J Exp Med* 1994, **179**, 643–649.
59. Botnick LE, Hannon EC, Hellman S. Multisystem stem cell failure after apparent recovery from alkylating agents. *Cancer Res* 1978, **38**, 1942–1947.
60. Hellman S, Botnick LE. Stem cell depletion. An explanation of the late effects of cytotoxins. *Int J Radiat Oncol Biol Phys* 1977, **2**, 181–184.
61. Tubiana M, Dutreix J, Wambersie A, Bewley D. *Introduction to Radiobiology*. London, Taylor and Francis, 1990.
62. Lord BI, Marshall E, Woolford LB, *et al.* Protection *in vivo* by BB10010 (MIP-1alpha) against repeated treatments with non-cycle-active cytotoxic agents. Sub-lethal irradiation. Submitted.
63. Paukovits WR, Laerum OD. Isolation and synthesis of a haemoregulatory peptide. *Zeitschrift fur Naturforschung* 1982, **37**, 1297–1300.
64. Laerum OD, Paukovits WR. Inhibitory effects of synthetic pentopeptide on haemopoietic cells *in vitro* and *in vivo*. *Exp Haematol* 1984, **12**, 7–17.
65. Paukovits WR, Guigon M, Binder KA, Hergl A, Laerum OD, Schulte-Hermann R. Prevention of haematotoxic side effects of cytostatic drugs in mice by a synthetic hemoregulatory peptide. *Cancer Res* 1990, **50**, 328–332.
66. Paukovits WR, Moser M, Binder K, Paukovits JB. Protection from arabinofuranosylcytosine and n-mustard induced myelotoxicity using hemoregulatory peptide pGlu-Glu-Asp-Cys-Lys monomer and dimer. *Blood* 1991, 77, 1313–1319.
67. Laerum OD, Sletvold O, Erikson J, *et al.* The dimer of hemoregulatory peptide (HP5b) stimulates murine and human myelopoiesis *in vitro*. *Exp Haematol* 1988, **16**, 2274–2280.
68. Frindel E, Guigon M. Inhibition of CFU entry into cycle by a bone marrow extract. *Exp Haematol* 1977, 5, 74–76.
69. Carde P, Chastang C, Goncalves E, *et al.* Seraspenide (AcSDKP) etude on phase I-II d'un inhibiteur de l'haematopoiese protegeant de la toxicite de monchimiotherapies aracytine et ifosfamide. *CR Acad Sci Paris* 1992, **315**, 545–550.
70. Grillon C, Prieger K, Bakala J, *et al.* Involvement of thymosin Beta 4 and endopeptidase AspN in the biosynthesis of the tetrapeptide Ac-Ser-Asp-lys-Pro, a regulator of the haematopoietic system. *FEBS Lett* 1990, **274**, 30–34.
71. Wdzieczak-Bakala J, Fache MP, Lenfant M, Frindel E, Sainteny F. AcSDKP, an inhibitor of CFU-s proliferation is synthesised in mice under steady state conditions and secreted by bone marrow in long term culture. *Leukaemia* 1990, **4**, 235–237.
72. Cashman JD, Eaves AC, Eaves CJ. Evidence for an indirect mechanism mediating the inhibitory effect of the tetrapeptide AcSDKP on primitive human haematopoietic cell proliferation. *J Cell Biochem* 1992, **16c**(suppl.), A95.
73. Guigon M. Biological properties of a low molecular weight pluripotent stem cell inhibitor. In Najman A, Guigon M, NC Gorin, JY Mary eds. *The Inhibitors of Haematopoiesis* 251. Paris, John Libbey Eurotext, 1987, **162**, 241–251.
74. Guigon M, Bonnet D, Lemoine F, *et al.* Inhibition of human bone marrow progenitors by the synthetic tetrapeptide AcSDKP. *Exp Haematol* 1990, **18**, 1112–1115.
75. Bogden A, Carde P, Deschamps de Paillet E, *et al.* Amelioration of chemotherapy-induced toxicity by cotreatment with AcSDKP, a tetrapeptide inhibitor of haematopoietic stem cell proliferation. *Ann NY Acad Sci* 1991, **628**, 126–139.
76. Hamilton C, Bonnet D, Mary JY, Lenfant M, Najman A, Guigon M. Evidence that the tetrapeptide AcSDKP reduces the toxicity of 3'-azido-3'-deoxythimidine (AZT) on normal bone marrow progenitors. *Blood* 1992, **80** (suppl. 4), A335.
77. Dunlop DJ, Campbell SM, Holyoake T, Steward WP. Phase I/Ib study of seraspenide (AcSDKP) in patients with advanced solid tumours. *Br J Cancer* 1994, **69**, A26.
78. Roberts AB, Anzario MA, Wakefield LM, Roche NS, Stern DF, Sporn MB. Type B transforming growth factor: a bifunctional regulator of cellular growth. *Proc Natl Acad Sci USA* 1985, **82**, 119–123.
79. Migdalska A, Molineux G, Demuynck H, Evans GS, Ruscetti F, Dexter TM. Growth inhibitory effects of transforming growth factor -β *in vivo*. *Growth Factors* 1991, **4**, 239–245.

80. Massague J. The transforming growth factor B family. *Annu Rev Cell Biol* 1990, **6**, 5597–5641.
81. Molineux G, Migdalaska A, Haley J, Evans G, Dexter TM. Total marrow failure induced by pegylated stem cell factor administered before 5-fluorouracil. *Blood* 1994, **12**, 3491–3499.
82. Sonis St, Lindquist L, Van Vugt A, *et al.* Prevention of chemotherapy-induced ulcerative mucositis by transforming growth factor-β3. *Cancer Res* 1994, **54**, 1135–1138.
83. Potten CS. *Stem Cells: Their Identification and Characterisation.* Edinburgh, Churchill Livingstone, 1983, 155–233.
84. Parkinson EK, Graham GJ, Daubersies P, *et al.* Haemopoietic stem cell inhibitor (SCI/MIP-1alpha) also inhibits clonogenic epidermal keratinocyte proliferation. *J Invest Dermatol* 1993, **101**, 113–117.
85. Hakovita H, Vierula M, Wolpe SD, Parvinen M. MIP-1alpha is a regulator of mitotic and meiotic DNA synthesis during spermatogenesis. *Molec Cell Endocr* 1994, **99**, 119–124.
86. Lombard MN, Sotty D, Wdzieczak-Bakala J, Lenfant M. *In vivo* effect of the tetrapeptide. N-acetyl-Ser-Asp-Lys-Pro on the G1/S transition of rat hepatocytes. *Cell Tissue Kinet* 1990, **23**, 99–103.
87. Cashman JD, Eaves AC, Eaves CJ. Primitive neoplastic haemopoietic cells from patients with chronic myeloid leukaemia can ignore the reversible inhibitory action of the tetrapeptide AcSDKP. *Exp Haematol* 1992, **20**, A299.
88. Eaves CJ, Cashman JD, Wolpe SD, Eaves AC. Unresponsiveness of primitive chronic myeloid leukaemia cells to macrophage inflammatory protein 1alpha–an inhibitor of primitive normal haematopoietic cells. *Proc Natl Acad Sci USA* 1993, **90**, 12015–12019.
89. Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of haemopoietic stem cells *in vitro*. *J Cell Physiol* 1977, **91**, 335–341.
90. Eaves CJ, Cashman JD, Kay RJ, *et al.* Mechanisms that regulate the cell cycle status of very primitive haematopoietic cells in long term human marrow cultures. II Analysis of positive and negative regulators produced by stromal cells within the adherent layer. *Blood* 1991, **1**, 110–117.
91. Ferrajol A, Talpaz M, Zipf TF, *et al.* Inhibition of acute myeloid leukaemia progenitor proliferation by macrophage inflammatory protein-1alpha. *Leukaemia* 1994, **8**, 798–805.
92. Lord BI, Woolford LB, Wood LM, *et al.* Mobilisation of early haemopoietic progenitor cells with BB10010: a genetically engineered variant human macrophage inflammatory protein-1alpha. *Blood* 1995, **85**, 3412–3416.