Pegfilgrastim: using pegylation technology to improve neutropenia support in cancer patients

Graham Molineux[a]

Pegylation of a protein can improve not only its formulation properties, but also both its pharmacokinetic and pharmacodynamic performance. Pegfilgrastim was made by linking a 20-kDa polyethylene glycol molecule to filgrastim, producing a long-acting cytokine requiring less frequent dosing than its parent drug. This review describes the clinical development of pegfilgrastim, and discusses its potential benefits to patients and caregivers in the prophylaxis of chemotherapy-induced neutropenia. Pegfilgrastim has a longer half-life and slower elimination rate than filgrastim, resulting in an increased serum concentration over time. Serum levels of pegfilgrastim are maintained until after a chemotherapy-induced neutrophil nadir and then decline rapidly as the neutrophil count recovers, consistent with a neutrophil-mediated clearance mechanism. In two pivotal phase III studies of women receiving chemotherapy for breast cancer, a single injection of pegfilgrastim per chemotherapy cycle, dosed either by body weight (100 μg/kg) or as a fixed dose (6 mg), was comparable to daily filgrastim (5 μg/kg) for all efficacy parameters, including duration of severe neutropenia and depth of neutrophil nadir. Analysis of pooled data from these studies showed a significantly lower incidence of febrile neutropenia in patients receiving pegfilgrastim compared with filgrastim (11 versus 19%, $p < 0.05$), and a trend towards a lower risk of hospitalization and use of i.v. anti-infectives. The safety profiles of pegfilgrastim and filgrastim were similar. Pegfilgrastim given once per chemotherapy cycle is as effective and well tolerated as daily injections of filgrastim. With its more convenient dosing regimen, pegfilgrastim has the potential to improve quality of life and compliance in patients, and to be more cost effective. *Anti-Cancer Drugs* 14:259–264 © 2003 Lippincott Williams & Wilkins.

[a]Hematology/Research, Amgen, Thousand Oaks, CA, USA.

Correspondence to G. Molineux, Hematology/Research, Amgen, Thousand Oaks, CA 91320, USA.
Tel: +1 805 447-7818; fax: +1 805 49-7506;
e-mail: grahamm@amgen.com

Introduction

The attachment of a polyethylene glycol (PEG) molecule to a protein (pegylation) is an established method of improving the pharmacological and clinical properties of the conjugated agent [1–3]. Potential benefits include greater biological activity, longer circulating half-life and more stable plasma concentrations. Pegylated proteins also tend to be less immunogenic than their parent compounds. These properties can mean less frequent administration and reduced toxicity for a patient, with the potential for greater patient compliance and improved quality of life.

With improvements in pegylation technology, a number of agents involved in cancer therapy have been pegylated and are being investigated or have regulatory approval. Examples include pegylated interferon (IFN)-α2a, currently under investigation for the treatment of renal cell carcinoma [4], and pegylated liposomal doxorubicin, which is already approved by the US Food and Drug Administration for Kaposi's sarcoma and advanced ovarian cancer, and is now being investigated in other solid tumors [5–7]. Pegylated molecules have also been developed for use in therapy areas other than cancer; pegylated IFN-α2b has been approved in the US for hepatitis C.

Pegylation of filgrastim (Neupogen; r-metHuG-CSF) has led to the development of pegfilgrastim (Neulasta). Filgrastim, a recombinant, bacterially derived granulocyte colony stimulating factor (G-CSF), stimulates the maturation, differentiation and proliferation of neutrophils and neutrophil precursors, and has proven efficacy as prophylaxis for chemotherapy-induced neutropenia (CIN) and febrile neutropenia (FN). By reducing the duration of severe neutropenia, the incidence of FN and hospitalization, and the use of i.v. anti-infectives, filgrastim offers important patient and pharmacoeconomic benefits. However, filgrastim has a short serum half-life and must be given by daily injection. Pegfilgrastim was developed as a long-acting cytokine requiring once per chemotherapy cycle dosing.

Pegylation technology

PEG molecules are inert, pH-neutral, non-toxic, hydrophilic polymers consisting of repeating ethylene oxide

subunits with two terminal hydroxyl groups that can be chemically activated. Each subunit has a molecular weight of 44 Da. PEG chains may be linear or branched structures, produced by joining a number of chains with linkers such as lysine or triazine.

PEG molecules are usually covalently linked to proteins. Early coupling methods hampered the development of effective new agents [8] and resulted in low-molecular-weight conjugates that often had inferior biological activity to the parent drug or had no distinctive pharmacokinetic properties. The linking components such as triazine rings or labile ester bonds were unstable, toxic or immunogenic. High-molecular-weight PEG molecules, which might have been more effective in prolonging half-life, could not be used because large amounts of diol were produced during the conjugation process. Diol activation produces a dysfunctional contaminant that can cause cross-linking and aggregation.

Improved pegylation technology produces conjugates with strong linkages. These are resistant to side reactions and can withstand purification to remove diol contaminants, making it possible to use high-molecular-weight molecules. PEG molecules are commonly linked to the amino group of lysine or the N-terminal amino group of the protein, but attachment is also possible via other functional groups including free cysteines, oligosaccharides and alcohol groups [1]. A critical step in the development of pegfilgrastim was the development of site-directed conjugation [9]. By using specific reaction conditions the amino group of the N-terminal amino acid can be differentially targeted to yield a more consistent molecular structure. This allows site-directed attachment of PEG moieties engineered to obtain the required drug profile, i.e. prolonged serum residence time, without interfering with the biological action of the drug.

Molecular properties of pegylated proteins

The physicochemical properties of pegylated proteins generally differ from those of the parent molecule [1,3,8]. Pegylation may induce changes in conformation, electrostatic binding properties and hydrophobicity, resulting in steric interference that hinders receptor interaction. Care must be taken to maximize the benefits of pegylation without compromising therapeutic activity. The size of the PEG molecule, whether it is branched or linear, and the site of pegylation influence the functionality of the final pegylated agent. Attaching a very large PEG moiety or attaching PEG chains at multiple sites may interfere with biological activity, e.g. by reducing receptor affinity. Long-chain PEG molecules become hydrated in aqueous solution and move constantly, protecting the conjugated protein from enzymatic degradation. Branched PEG molecules generally offer greater protection due to higher steric hindrance, while low molecular weight PEG chains may not protect the protein at all.

The *in vitro* activity of a pegylated protein rarely predicts its *in vivo* activity, and is inversely related to the mass and number of PEG molecules linked to the protein [1,10]. Incubation times in *in vitro* assays are relatively short and any adverse effects of pegylation on receptor binding dominate apparent biological activity.

Conversely, *in vivo* activity generally increases with increasing molecular weight, as factors influencing exposure to the therapeutic agent become more critical [1,10]. Area under the time–concentration curve (AUC) and half-life both increase with molecular weight. These effects may be partly attributed to reduced enzymatic degradation, but also to decreased renal clearance from the increased molecular mass of pegylated proteins. Higher-molecular-weight PEG conjugates injected intramuscularly or s.c. are also absorbed more slowly, which has an impact on pharmacokinetic parameters.

Development of pegfilgrastim

Pegfilgrastim comprises a 20-kDa PEG molecule covalently conjugated to the N-terminal methionine residue of filgrastim. Pegfilgrastim was developed to produce a long-acting cytokine requiring less frequent dosing than its parent drug.

Filgrastim and pegfilgrastim were initially compared in both mice and healthy volunteers [11]. In hematopoietically normal animals, a single injection of pegfilgrastim increased both peak and duration of elevated absolute neutrophil count (ANC) in a dose-dependent manner, and effectively mobilized peripheral blood progenitor cells. Fluctuations in neutrophil count throughout the day were minimized after a single dose of pegfilgrastim compared with daily filgrastim. In mice with CIN, pegfilgrastim assisted neutrophil recovery over a similar period to filgrastim. In healthy volunteers, ANC levels increased above baseline for around 9–10 days in a dose-dependent manner following a single injection of pegfilgrastim (30–300 µg/kg). The median time to ANC maximum was between 2.5 days (30 µg/kg) and 5 days (300 µg/kg).

Pharmacokinetics of pegfilgrastim

A randomized, open-label, dose-escalation study evaluated the pharmacokinetics, efficacy and safety of pegfilgrastim compared with filgrastim in 13 patients with non-small cell lung cancer (NSCLC) receiving carboplatin (dosed to AUC 6) and paclitaxel (225 mg/m^2) [12]. Patients received either a single s.c. injection of pegfilgrastim (30, 100 or 300 µg/kg) 14 days before chemotherapy and 24 h after completion of the chemotherapy cycle or five daily s.c. injections of filgrastim

(5 µg/kg) 14 days before chemotherapy and daily injections of filgrastim (5 µg/kg) starting 24 h after completion of the chemotherapy cycle until ANC $\geq 10 \times 10^9$/l.

Before chemotherapy, both filgrastim and pegfilgrastim induced a rapid rise in ANC. This response was sustained for longer in pegfilgrastim- than in filgrastim-treated patients, in whom the ANC decreased to pretreatment levels soon after filgrastim was discontinued. Pegfilgrastim produced a dose-dependent duration of response and peak ANC. Following 100 µg/kg pegfilgrastim, the peak ANC was similar to that in filgrastim-treated patients (approximately 32×10^9/l).
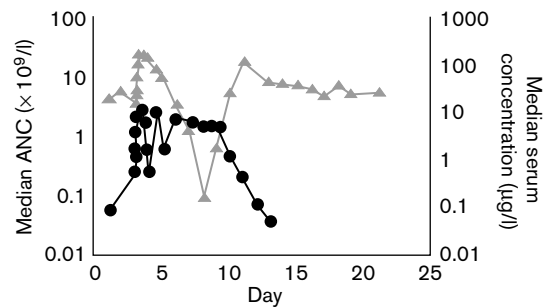
After chemotherapy, a similar pattern of ANC recovery was observed with once-per-cycle pegfilgrastim and daily filgrastim-treated patients. The median ANC nadir was around 0.1×10^9/l for patients receiving 30 µg/kg pegfilgrastim and daily filgrastim, and higher (around 0.7×10^9/l) in patients receiving 100 or 300 µg/kg pegfilgrastim. Neutrophil recovery was similar in all patients. Both agents resulted in comparable $CD34^+$ cell mobilization, although the preclinical studies had shown that pegfilgrastim was more effective at mobilizing $CD34^+$ cells than filgrastim. Adverse events were similar in all treatment groups.

The pharmacokinetics of pegfilgrastim were non-linear and dose-dependent. With increasing doses of pegfilgrastim, the maximum plasma concentration and the AUC increased while clearance decreased. Pegfilgrastim levels remained elevated for longer than filgrastim. Table 1 shows the values of pharmacokinetic parameters in patients receiving 100 µg/kg pegfilgrastim.

Dose finding

A phase II study in women with breast cancer evaluated the optimal dose of pegfilgrastim to provide both neutrophil support and a tolerability profile comparable to that of filgrastim [13]. Chemotherapy comprised four cycles of doxorubicin (60 mg/m^2) and docetaxel (75 mg/m^2) every 21 days. One hundred and fifty-four women were randomized to one of three doses of pegfilgrastim (30, 60 or 100 µg/kg) as a single s.c. injection, or filgrastim

Table 1   Pharmacokinetics of pegfilgrastim and filgrastim [13]

Parameter	Pegfilgrastim 100 µg/kg (n=3) [median (range)]	Filgrastim 5 µg/kg/day (n=3) [median (range)]
C_{max} (ng/ml)	114 (58.1–203)	10.7 (9.15–15.5)
t_{max} (h)	72.0 (24.0–96.0)	8.0 (8.0–8.0)
$t_{1/2}$ (h)	33.2 (30.3–53.8)	3.37 (3.10–4.84)
$AUC_{0-\infty}$ (ng/ml/h)	7150 (6320–24100)	126 (81.9–155)
CL/F (ml/h/kg)	14.0 (4.15–15.8)	39.6 (32.3–61.1)

C_{max}=maximum plasma concentration; t_{max}=time to C_{max}; $t_{1/2}$=terminal elimination half-life; $AUC_{0-\infty}$=area under the plasma concentration–time curve from time zero to infinity; CL/F=apparent serum clearance after s.c. administration.

Fig. 1

Semi-logarithmic plots of median ANC (triangles) and median serum filgrastim concentration (5 µg/kg/day; n=3) (circles) over time. Filgrastim serum levels fluctuate with daily dosing. Adapted with permission from [12].

Table 2   Incidence and duration of chemotherapy-induced, grade 4 neutropenia in women with breast cancer following pegfilgrastim or filgrastim [13]

Parameter[a]	Filgrastim	Pegfilgrastim		
	5 µg/kg	30 µg/kg	60 µg/kg	100 µg/kg
Grade 4 neutropenia incidence (% patients)	76	95	90	74
mean duration (days)	1.6	2.7	2.0	1.3
duration 0–2 days (% patients)	88	37	67	87
duration ≥ 3 days (% patients)	12	63	33	13
Mean time to ANC recovery (days)[b]	9.4	11.0	10.3	9.5

[a]All data collected during cycle 1 of chemotherapy.
[b]Time to ANC $\geq 2 \times 10^9$/l.

(5 µg/kg) as daily s.c. injections for 14 days or until ANC $\geq 10 \times 10^9$/l, both starting 24 h after chemotherapy was completed in each cycle. From the total group, 152 and 150 patients were evaluable for safety and efficacy, respectively.

The incidence of grade 4 neutropenia during cycle 1 was similar in patients who received a single 100 µg/kg injection of pegfilgrastim and daily 5 µg/kg filgrastim injections. Compared with filgrastim, a single 100 µg/kg dose of pegfilgrastim resulted in a similar or shorter duration of grade 4 neutropenia in every chemotherapy cycle and a similar ANC profile, including time to recovery (defined as ANC $\geq 2 \times 10^9$/l) (Fig. 1 and Table 2).
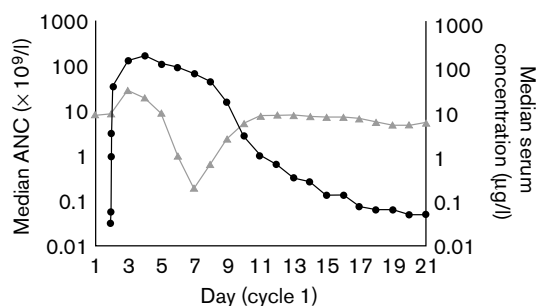
Pegfilgrastim pharmacokinetics were consistent with those described above: non-linear, dose dependent and

Fig. 2

Semi-logarithmic plots of median ANC (triangles) and median serum pegfilgrastim concentration (100 μg/kg/day; $n = 46$) (circles) over time. Pegfilgrastim clears as neutrophil levels return to normal. Adapted with permission from [13].

Table 3   The effect of pegfilgrastim compared with filgrastim on CIN in patients with NHL or Hodgkin's disease [16]

Parameter	Filgrastim 5 μg/kg	Pegfilgrastim 100 μg/kg
Incidence of grade 4 neutropenia (%)	68	69
Mean duration of severe neutropenia (days)	2.4	2.8
Median time to ANC recovery (days)[a]	15	16
Incidence of febrile neutropenia (%)[b]	19	21

[a]Time to ANC $\geq 2 \times 10^9$/l, in cycle 1.
[b]Cumulative incidence in cycles 1 and 2.

ANC dependent. The pegfilgrastim serum concentration was maintained until after the neutrophil nadir and then declined rapidly as the ANC recovered, consistent with a neutrophil-mediated clearance mechanism. The safety profiles of pegfilgrastim and filgrastim were similar.

These results led to the conclusion that 100 μg/kg pegfilgrastim was the dose with a profile most comparable to 5 μg/kg filgrastim and so it was selected for further investigation in phase III trials.

More recently, a biomathematical model was developed to describe the pharmacokinetics and pharmacodynamics of single-dose pegfilgrastim and daily filgrastim in patients with NSCLC [14,15]. Taking into account G-CSF receptor-dependent clearance of pegfilgrastim, myelosuppressive effects of chemotherapy and granulo-poietic effects of pegfilgrastim, this model also predicted that 100 μg/kg pegfilgrastim would be the dose most similar to 5 μg/kg/day filgrastim (Fig. 2).

Phase II study in patients with non-Hodgkin's lymphoma (NHL) or Hodgkin's disease

A randomized, open-label phase II trial compared the safety and efficacy of a single s.c. dose of pegfilgrastim (100 μg/kg) with daily s.c. doses of filgrastim (5 μg/kg) following etoposide, methylprednisolone, cisplatin and cytarabine (ESHAP) chemotherapy in 60 patients with relapsed or refractory NHL or Hodgkin's disease [16]. Cytokines were given 24 h after chemotherapy in each cycle and filgrastim was continued daily for 12 days or until ANC $> 2 \times 10^9$/l. A single dose of pegfilgrastim was as effective as daily injections of filgrastim with respect to the incidence and duration of severe neutropenia (DSN), incidence of FN, and median time to neutrophil recovery (Table 3). There were no differences in the incidence, duration and severity of bone pain or other adverse events between the two treatment groups.

Phase III studies in patients with breast cancer

Two large, double-blind, randomized, phase III trials compared the equivalence of pegfilgrastim and filgrastim in patients with breast cancer treated with doxorubicin (60 mg/m^2) and docetaxel (75 mg/m^2) given every 21 days for four cycles. The primary efficacy endpoint was DSN (ANC $< 5 \times 10^9$/l) in cycle 1. One trial dosed pegfilgrastim by weight (100 μg/kg s.c.) [17], while the second used a fixed, 6 mg s.c. dose of pegfilgrastim [18]; both were compared with daily s.c. filgrastim (5 μg/kg).

In the by-weight dosing trial, 154 patients were randomized to once-per-chemotherapy-cycle pegfilgrastim and 156 patients to daily filgrastim [17]. Patients in each group were well matched for stage of disease and previous treatment. The mean DSN during cycle 1 was 1.7 and 1.8 days for pegfilgrastim and filgrastim-treated patients (> 0.5), respectively, with a mean difference of 0.03 days and a two-sided 95% confidence interval (CI) of (-0.36, 0.30). In cycles 2–4, the DSN was significantly shorter in the pegfilgrastim group than in the filgrastim group (0.6–0.9 versus 1.1–1.3; $p \leq 0.025$). The greatest incidence of severe neutropenia occurred during the first cycle of chemotherapy and was similar in both treatment groups. This similarity continued in all subsequent cycles, although the incidence tended to be lower in the pegfilgrastim group than in the filgrastim group.

Similar results were obtained in the fixed-dose study [18]. The mean DSN during cycle 1 was 1.8 and 1.6 days, respectively, in 77 pegfilgrastim and 75 filgrastim patients who were evaluable. The mean difference in DSN between filgrastim and pegfilgrastim was 0.23 days, with a two-sided 95% CI of (-0.15, 0.63 days). Pegfilgrastim showed comparable efficacy to daily filgrastim when administered as a 6 mg fixed dose, once per chemotherapy cycle, over a broad range of patient weights. The fact that fixed-dose pegfilgrastim did not compromise efficacy in patients weighing more than 80 kg or tolerability in those weighing less than 60 kg further simplifies the use of pegfilgrastim.

The incidence of FN was a prospectively defined, clinically relevant endpoint in both studies. When pegfilgrastim was dosed by weight, the incidence of FN over all chemotherapy cycles was significantly lower than with filgrastim (9 versus 18%; $p = 0.029$) [17]. There was a similar trend favoring pegfilgrastim when pegfilgrastim was given as a fixed dose (FN incidence 13 versus 20%; $p =$ not significant) [18].

Analysis of pooled data from both phase III trials revealed a significantly lower incidence of FN for patients receiving pegfilgrastim than for those receiving filgrastim (11 versus 19%; $p < 0.05$), with a significantly shorter duration ($p < 0.05$) [19]. There was also a trend towards lower risk of hospitalization and use of i.v. anti-infectives in patients receiving pegfilgrastim. The authors suggest that these findings may be related to the sustained and relatively steady serum levels of pegfilgrastim compared with the cyclical variations seen with daily filgrastim injections.

'Self-regulating' mechanism of pegfilgrastim clearance

Filgrastim appears to be eliminated from the body by two mechanisms. Clearance occurs primarily via the kidneys and secondarily via neutrophil receptor-mediated clearance. The addition of the PEG moiety to filgrastim makes the resulting pegfilgrastim molecule too large for renal clearance, leaving neutrophil receptor-mediated clearance as the primary mechanism of removal [20]. In neutropenic patients this route of elimination is minimized. During recovery, the emergence of newly formed neutrophils into the circulation increases the rate of pegfilgrastim clearance. Mature neutrophils are relatively poor at clearing pegfilgrastim, whereas younger neutrophils have higher receptor number and possibly receptor density, and are more effective at clearing pegfilgrastim. Pegfilgrastim serum levels therefore remain elevated until ANC recovers, at which point they begin to decline, thereby producing a 'self-regulating' mechanism of clearance (Fig. 1).

Clinical benefits of pegfilgrastim

Pegfilgrastim achieves neutropenia prophylaxis that is at least equivalent to that of filgrastim. However, because pegfilgrastim serum levels are sustained, only one dose is needed per chemotherapy cycle in contrast to daily injections required for filgrastim. Less frequent dosing simplifies neutropenia management and may have important benefits for patient quality of life [16]. Fixed dosing further simplifies the management of neutropenia as it minimizes potential dosing errors.

One of the major costs of G-CSF in hospital patients is the need for daily blood sampling to monitor neutrophil levels [21]. The once-per-cycle administration of pegfilgrastim and its novel neutrophil-mediated clearance eliminate this requirement. Less frequent dosing and reduced neutrophil monitoring may provide an additional pharmacoeconomic rationale for using pegfilgrastim.

The significant reduction in incidence and duration of FN is an important clinical benefit of pegfilgrastim that could lead to improved patient quality of life and reduce the risk of contracting potentially life-threatening infection. The trend for reduced hospitalization and use of i.v. anti-infectives suggests that using pegfilgrastim instead of filgrastim may result in cost savings in the overall management of CIN.

Conclusions

The development of pegfilgrastim by adding a PEG molecule to filgrastim has produced a molecule with a longer half-life and reduced rate of elimination, leading to sustained plasma levels. As a result, pegfilgrastim has a more convenient dosing schedule than filgrastim that does not compromise efficacy or safety.

Pegfilgrastim may provide other important benefits that have not yet been fully explored, including cost savings and improved quality of life for patients due to the once-per-chemotherapy-cycle dosing schedule, less frequent neutrophil monitoring and lower incidence of FN.

Pegfilgrastim is an excellent example of how pegylation technology can improve the properties of a therapeutic agent, increasing the choice available to health care providers and offering real clinical benefits. Pegfilgrastim undoubtedly has an important role to play in the management of CIN.

References

1 Bailon P, Berthold W. Polyethylene glycol-conjugated pharmaceutical proteins. *Pharm Sci Technol Today* 1998; **1**:352–356.
2 Crawford J. Clinical benefits of pegylated proteins in oncology. *Cancer Treat Rev* 2002; **28** (**suppl A**):1–2.
3 Reddy KR. Controlled-release, pegylation, liposomal formulations: new mechanisms in the delivery of injectable drugs. *Ann Pharmacother* 2000; **34**:915–923.
4 Motzer RJ, Thompson J, Gurney H, Selby P, Figlin R, Negrier S, et al. Phase II trial of branched pegylated (40 kDa) interferon α-2a for patients with advanced renal cell carcinoma (RCC). *Proc Am Soc Clin Oncol* 2001; **20**:abstr 716.
5 Coukell AJ, Spencer CM. Polyethylene glycol–liposomal doxorubicin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in the management of AIDS-related Kaposi's sarcoma. *Drugs* 1997; **53**:520–538.
6 Judson I, Radford JA, Harris M, Blay J-Y, van Hoesel Q, le Cesne A, et al. Randomised phase II trial of pegylated liposomal doxorubicin (Doxil/Caelyx) versus doxorubicin in the treatment of advanced or metastatic soft tissue sarcoma: a study by the EORTC Soft Tissue and Bone Sarcoma Group. *Eur J Cancer* 2001; **37**:870–877.
7 Keller AM, Mennel RG, Nabholtz JM, Georgoulias V, Emanuel DJ, Tendler CL. Phase III trial of pegylated liposomal doxorubicin (Caelyx/Doxil) for the treatment of patients with advanced breast cancer who have failed a prior taxane-containing regimen. *Proc Am Soc Clin Oncol* 2001; **20**:abstr 115.
8 Molineux G. Pegylation: engineering improved pharmaceuticals for enhanced therapy. *Cancer Treat Rev* 2002; **28** (**suppl 1**):13–16.

9   Kinstler O, Molineux G, Treuheit M, Ladd D, Gegg C. Mono-N-terminal poly(ethylene glycol)–protein conjugates. *Adv Drug Deliv Rev* 2002; **54**:477–485.

10   Bowen S, Tare N, Inoue T, Yamasaki M, Okabe M, Horii I, *et al.* Relationship between molecular mass and duration of activity of polyethylene glycol conjugated granulocyte colony-stimulating factor mutein. *Exp Hematol* 1999; **27**:425–432.

11   Molineux G, Kinstler O, Briddell B, Hartley C, McElroy P, Kerzic P, *et al.* A new form of filgrastim with sustained duration *in vivo* and enhanced ability to mobilize PBPC in both mice and humans. *Exp Hematol* 1999; **27**:1724–1734.

12   Johnston E, Crawford J, Blackwell S, Bjurstrom T, Lockbaum P, Roskos L, *et al.* Randomized, dose-escalation study of SD/01 compared with daily filgrastim in patients receiving chemotherapy. *J Clin Oncol* 2000; **18**:2522–2528.

13   Holmes FA, Jones SE, O'Shaughnessy J, Vukelja S, George T, Savin M, *et al.* Comparable efficacy and safety profiles of once-per-cycle pegfilgrastim and daily injection filgrastim in chemotherapy-induced neutropenia: a multicenter dose-finding study in women with breast cancer. *Ann Oncol* 2002; **13**:903–909.

14   Roskos LK, Cheung EN, Schwab G, *et al.* A cytokinetic model of granulopoiesis and the homeostatic regulation of pegfilgrastim clearance in patients with non-small cell lung cancer. *Blood* 2003; in press.

15   Roskos LK, Yang B, Scwab G, *et al.* A cytokinetic model of r-metHuG-CSF-SD/01 (SD/01) mediated granulopoiesis and the self-regulation of SD/01 elimination in non-small cell lung cancer (NSCLC) patients. *Blood* 1998; **92**:507 (abstr 2085).

16   Vose JM, Crump M, Lazarus H, *et al.* Single dose pegfilgrastim (SD/01) is as effective as daily filgrastim following ESHAP chemotherapy for subjects with non-Hodgkin's lymphoma or Hodgkin's disease: results of a randomized, open-label study. *J Clin Oncol* 2003; in press.

17   Holmes FA, O'Shaughnessy J, Vukelja S, Jones SE, Shogan J, Savin M *et al.* Blinded, randomized, multicenter study to evaluate single administration pegfilgrastim once per cycle versus daily filgrastim as an adjunct to chemotherapy in patients with high-risk stage II or stage III/IV breast cancer. *J Clin Oncol* 2002; **20**:727–731.

18   Green MD, Koelbl H, Baselga J, Galid A, Guillem V, Gascon P, *et al.* A randomized, double-blind, phase 3 study of fixed-dose, single-administration pegfilgrastim vs filgrastim in patients receiving myelosuppressive chemotherapy. *Ann Oncol* 2003; **14**:29–35.

19   Siena S, Piccart MJ, Holmes FA, Glaspy J, Hackett J, Renwick J. A single dose of pegfilgrastim per chemotherapy cycle reduces the incidence and duration of febrile neutropenia compared with daily filgrastim: a meta-analysis of two phase 3 randomised trials in patients with stage II–IV breast cancer. *Oncol Rep* 2003; in press.

20   Yowell SL, Blackwell S. Novel effects with polyethylene glycol-modified pharmaceuticals. *Cancer Treat Rev* 2002; **28** (**suppl A**):3–6.

21   Annemans L, Vanoverbeke N, Caekelbergh K, Van Belle S, Cocquyt V, Standaert B. Estimating and comparing resource use and cost of neutropenia management using G-CSF in chemotherapy with the activity-based costing (ABC) method in three settings. *Value in Health* 2001; **4**:abstr PM16.