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Pegfilgrastim – designing an improved form of rmetHuG-CSF

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Abstract

rmetHuG-CSF is the recombinant version of natural granulocyte colony-stimulating factor, the dominant stimulator in the production of neutrophilic leukocytes (neutrophils). Neutrophils represent the first line of defense against invading pathogens and when neutrophil numbers are suppressed by cancer chemotherapy, patients become liable to life-threatening infections.
The clearance of rmetHuG-CSF is effected by a combination of neutrophil mediated degradation and renal filtration. Site-directed addition of a single, linear PEG molecule yielded a form of G-CSF (pegfilgrastim) that was shown to be resistant to renal elimination yet remained sensitive to neutrophil-mediated destruction. This semi-synthetic cytokine drug can persist in the plasma for extended periods in neutropenic conditions, yet is cleared rapidly when neutrophils recover. This lends a degree of automation to the therapeutic control of neutrophil numbers which has been exploited in clinical practice since its approval for human use in 2002.

Introduction

Natural Granulocyte Colony-Stimulating Factor (G-CSF) is a circulating glycoprotein that regulates neutrophil production and activity. Neutrophils normally comprise around 30% of leukocytes and are major effectors of innate immunity. Neutrophils remain in the blood only a matter of hours so they need to be replaced by rapid proliferation of their precursors in the bone marrow (Tab. 1). This high turnover rate makes neutrophil production susceptible to cancer chemotherapy and neutropenia (lack of neutrophils) following chemotherapy leaves many patients prone to infection and hospitalization. The use of G-CSF to stave off such complications has become widespread practice over the last 20 years. This was made possible by the cloning and characterization of recombinant human G-CSF (rHuG-CSF) in the early 1980s [1–3] culminating in the expression of r-metHuG-CSF in *E. coli*, trials in humans and eventually approval of Filgrastim® for administration to US patients in 1991 [4–7]. Alternative forms of G-CSF also exist, among them a form expressed in Chinese hamster ovary (CHO) cells (lenograstim; Granocyte®), which is glycosylated and lacks the N-terminal methionine required in the *E. coli*-derived version [8] another form with a deliberately mutated amino acid sequence [9] and several follow-on versions (Tevagrastim®, Ratiograstim®, ratiopharm Filgrastim® and Biograstim®).

Table 1. Production rate of human blood cells

Blood cell type	Concentration of cells in blood	Total cells in blood (5L blood volume)	Lifespan of cells	Production rate
Neutrophils	3×10^3/μL	15×10^9	8 h	4.5×10^{10}/day
Erythrocytes	5×10^6/μL	25×10^{12}	120 days	21×10^{10}/day

Blood cell numbers are stable in normal individuals. The lifespan of each of the cell types is quite different, but to maintain stable circulating numbers cell production must precisely balance cell loss. This process is under the control of hormone-like cytokines, in the case of neutrophils, granulocyte colony-stimulating factor, and in the case of erythrocytes, erythropoietin. Production rates can be increased dramatically or reduced to almost zero in response to physiological demand.

Initially, administration of G-CSF was for the treatment of cancer chemotherapy-induced neutropenia (CIN) and the prevention of associated infections, but over the years the indications have been broadened to include use in severe congenital neutropenia, AIDS, aplastic anemia and myelodysplastic syndromes. In addition, the serendipitous finding that G-CSF can 'mobilize' large numbers of transplantable 'stem cells' to the blood has been exploited in both cancer patients and normal donors [10, 11]. In this setting G-CSF can cause peripheral blood progenitor cells (PBPC) to move to the blood, where they may be harvested to allow remedial treatment of damaged or diseased bone marrow. To date over 4 million patients have received G-CSF for various indications, with the only major reported side effect being bone pain – perhaps part of the bone marrow's normal response to G-CSF.

rHuG-CSF has to be injected into the body because it is degraded in the stomach and is too large to pass unaided through the skin. Continuous infusion is the most effective way to administer G-CSF, followed by twice daily injection, and daily administration; from there effectiveness is yet further reduced as injections are more widely spaced. The requirement for frequent G-CSF injections stems from its rapid clearance from the body mainly via the kidney but also due to neutrophil-mediated processes. Clinical experience has shown that G-CSF can be administered to patients intravenously (IV) [5, 6], subcutaneously (SC) [12] or intramuscularly [13] and in all cases the neutrophil response is similar. After IV injection G-CSF levels increase within a matter of minutes [5, 6] though SC administration also shows very rapid absorption [7]. Clearance of G-CSF from the body is very fast as illustrated by a serum half-life of between 1–2 h in several tested species [14–16]. Normal humans clear G-CSF with a half-life of less than 2 h [17], but neutropenic patients take almost 5 h to clear half the drug concentration – *prima facie* evidence that G-CSF half-life is related inversely to absolute neutrophil count (ANC). This suggests that neutrophils themselves may play a role in the clearance of G-CSF – not an unprecedented suggestion since the elimination of hematopoietic cytokines by the products of those cytokines' action has been proposed for several important regulators [18–25]. In this model (Fig. 1) at least one cytokine

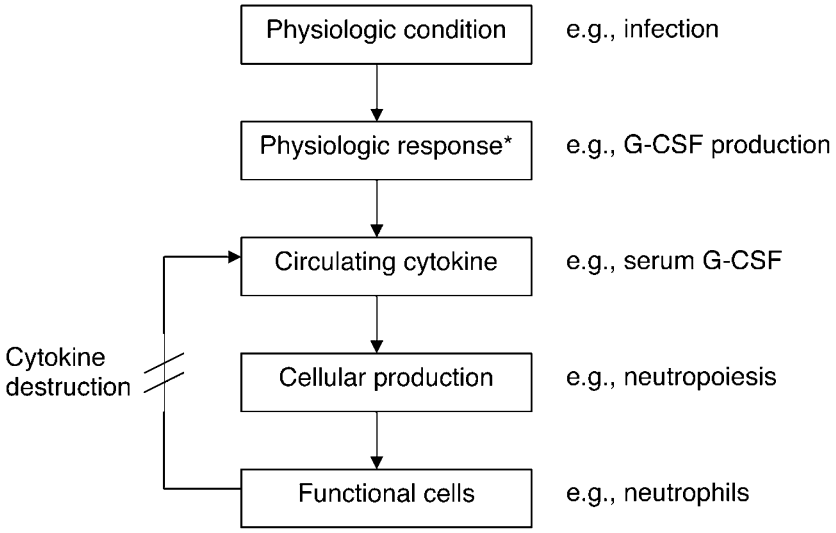

Figure 1. Autoregulation of lineage specific cytokine levels by terminally differentiated cells of the affected cellular lineage. * some cytokines are inducible (e.g., G-CSF), others are produced constitutively (e.g., thrombopoietin, the main stimulator of platelet production), the model is unaffected.

involved in the control of blood cell development would be a lineage specific regulator, i.e., one that shows high fidelity for that single cellular lineage, for example thrombopoietin for platelets or G-CSF for neutrophils. The terminal cell type of that lineage would then control the circulating levels of the regulator possibly by receptor-mediated internalization and degradation. In the specific case of G-CSF the reciprocal relationship between G-CSF levels and neutrophil counts has been reported numerous times [26–29], and the ability of neutrophils to destroy G-CSF *in vitro* [30] has also been documented. Neutrophil-mediated clearance of G-CSF is not the only contributor to its rapid removal, indeed an alternative route – renal excretion, may account for the majority of clearance in some situations [31, 32]. Renal loss is of sufficient magnitude that in order to maintain effective serum levels even in conditions of absolute neutropenia, daily injections are required.

To summarize the effect of these two modes of clearance consider the fate of the first of a series of filgrastim injections. If this injection were made to a normal individual, with normal ANC, then as injected dose and serum concentration increased, the drug would be absorbed by the neutrophil population (and lost from both measurements of serum G-CSF and prevented from influencing neutropoiesis). When that compartment was saturated, the excess would then be circulating in the serum where it could stimulate neutrophil production, but would be eliminated from the serum via the kidney with a half-life of about 5 h. Should the initial injection have been made into a neutropenic individual then none of the drug would be lost to the neutrophil pool and all

would be subject to renal elimination with a 5 h half-life, stimulating neutrophil production in the meantime. In considering the fate of subsequent injections account must be taken of the accumulating response to G-CSF, i.e., ascending ANC. As neutrophil numbers increase over time the persistence of each injection would be progressively shorter as more drug was absorbed and destroyed by the ANC pool; indeed, this was what was actually observed in early clinical studies [25]. Thus the predominant clearance pathway for G-CSF will depend on the saturable (and therefore non-linear) ANC-related route and the linear renal pathway which will in turn depend on the response to the drug (ANC increases as G-CSF has its effects). So unmodified G-CSF displays a degree of 'self regulation' – it induces the means of its own destruction – but this process is of relatively minor importance because most of the drug is lost through the relentless renal process.

These considerations suggested a strategy that might be employed to design a successor to filgrastim – it was reasoned that the contribution of the two routes of clearance could be manipulated independently to engineer a derivative of G-CSF that resisted renal clearance yet retained sensitivity to neutrophil-mediated destruction. In considering the properties of a next generation therapeutic as many of the desirable properties listed below should be built in to the molecule:

1) The safety record of the parent drug must not change, i.e., no non-G-CSFR mediated effects and no increase in antigenicity would be tolerated
2) The formulation properties of the drug (stability, solubility, etc.) should be at least maintained
3) The persistence of the drug in the body must be increased to cover, if possible, a complete cycle of chemotherapy from a single injection
4) The 'exposure profile' should be optimized, i.e., more drug should be provided at the time it is most needed, less when less is needed.

To deliver as many of these properties as possible filgrastim was retained at the core of the molecule as data to date would suggest that this protein has a single cellular receptor, and that in turn that receptor has but a single ligand – retaining this core would reduce the potential for introduction of new off-target activities. The stability of filgrastim is good as formulated, but G-CSF is inherently unstable at physiological pH, temperature and salt concentration – this was considered a relatively easy profile upon which improvements could be made. An increase in size presented a simple and proven method to evade renal clearance, but absent detailed knowledge of the optimum exposure profile for G-CSF was not known whether this change would in itself be sufficient to satisfy the longevity requirements. In considering what was known about the optimum exposure profile, inferences could be drawn from the literature; it is established 1) that continuous infusion offers superior efficacy [25, 33, 34], 2) that increased serum levels provoke a greater response [4] and 3) that early provision of G-CSF is important to maximize benefit [26].

Strategies for the improvement of rmetHuG-CSF

Two broad strategies were considered to improve G-CSF; sustained release and sustained duration. The physicochemical properties of G-CSF do not lend themselves well to the fabrication of a depot formulation. Neither is it an easy protein to deliver through skin or via the gastrointestinal tract. These delivery approaches are feasible to a degree but are less readily controlled than modification of the residence time of G-CSF in the circulation.

A sustained duration form of G-CSF that could sustain its effects for four weeks would need to be administered at relatively high doses (Filgrastim is administered at 5 μg/kg/day, so a 70 kg patient would require 9.8 mg for 28 days treatment assuming the introduction of zero inefficiency). Such a large amount of drug would unavoidably offer front-loading when administered as a single injection. Also, if this injection were administered shortly after chemotherapy then the resulting high serum concentration would coincide with the time at which the marrow required maximum impetus to launch recovery.

Protein therapeutics have been modified in various ways to extend their persistence in the body. Recently notable has been the success of glycoengineering an analog of erythropoietin to prolong its half-life [35]. In this exercise understanding the role carbohydrate played in controlling the elimination of erythropoietin was extended to engineer a hyperglycosylated variant with three times the residence time in the body [36, 37]. Though natural G-CSF is a glycoprotein (with a single O-linked carbohydrate on threonine 133) a comparison of two forms; one of which is produced in eukaroytic cells and glycosylated while filgrastim is made in prokaryotic cells and has no attached sugars, revealed their pharmacokinetics to be identical. This would suggest that the carbohydrate component is entirely optional for activity and further suggests that this may not be the most rewarding pathway to an extended duration derivative. Having thus eliminated glycoengineering from our considerations what other strategies are likely to work? A serum albumin conjugate of G-CSF has been discussed [38] and poly[ethylene glycol] (PEG) derivatives have been known for some years [16, 39–41].

As discussed above, a detailed consideration of the dual routes of elimination of G-CSF suggested that separation of these processes might lead to a new and particularly useful form. G-CSF is normally administered to patients who are neutropenic following cancer chemotherapy. In neutropenia only the kidney effects G-CSF clearance. The development of a form of G-CSF that could resist renal clearance, yet retain neutrophil-mediated clearance held the promise of enhancing the self-regulation which was already a feature of the parent molecule.

PEGylation

Several drugs; enzymes [42, 43], interferons ([44, 45], see elsewhere this volume) and cytokines [46–49], have been developed by covalently attaching

PEG because it confers benefits such as reduced immunogenicity, prolonged residence time or improved formulation properties [42, 43, 50–52]. In general the advantages gained by the protein conjugation are the properties of PEG itself, especially the unique ability of PEG to occupy a disproportionately large volume in aqueous solution. As an increase in size was one of the main targets of filgrastim derivitization, PEG was thought to be uniquely suitable for our purposes.

Several different ways of attaching PEG to proteins have been reported [47, 53], and most are similar in that they rely on the nucleophilic attack of amino groups (or other active protein components) on the terminal ethylene glycol group of PEG. In most cases such a reaction can be shown to yield reproducible forms with consistent location and number of attached PEGs [54]. Though the actual sites of attachment cannot be realistically determined in advance and are in effect controlled by the chemistry, precisely this approach has proven useful for two FDA approved PEG-enzyme conjugates [42, 43]. Considering a similar approach for a cytokine presents a different set of constraints. Cytokines tend to be large molecules with complex three-dimensional structure, and their receptors also tend to be similarly large and complex. Employing a non-selective PEGylation strategy in such a circumstance is likely to yield a suboptimal product. This is because of the contrasting effects of increasing serum residence time on one hand, but on the other hand lowering the affinity of the ligand/receptor interaction, probably by steric hindrance. The interplay of these factors will determine the usefulness of any derivative and selection of the final candidate tends to be a semi-empirical process. To avoid the vagaries of non-directed PEGylation several site-directed approaches have been developed. These techniques have been used for, e.g., topographical mapping of attachment sites [51]. In these cases, PEGylation can be targeted to, say, specific lysines in the amino acid sequence, but general sites of attachment can also be targeted such as the N- or C-terminus.

Targeting specific amino acids, most commonly lysine, can be useful especially if a limited number of such lysines are in desirable locations within the protein. If the sites at which such amino acids are found are not deemed desirable then lysines may be substituted for less reactive arginines or new lysines may be inserted at the appropriate site. However, a large protein may have several potential attachment sites that would require extensive re-engineering to remove from the molecule. These numerous sites would tend to produce a multi-PEGylated protein though some have been shown to retain substantial biological activity. However, in the case of G-CSF, the four lysines found throughout the molecule tend to be in regions that are not good target areas. A second site-directed approach mediated via specific amino acids is targeting the thiols of cysteine residues. Again cysteines can be introduced or removed to lend a protein to this type of chemistry. However, the three dimensional configuration of G-CSF is stabilized by several disulfide bonds between cysteines and derivitization of any of them may upset the structure leading to potential affects on activity and immunogenicity (Fig. 2).

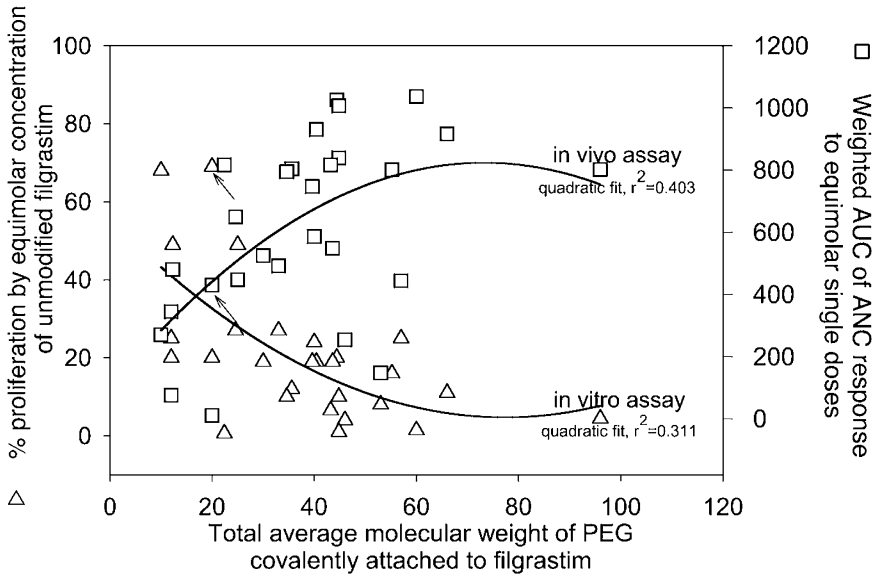

Figure 2. The activity of various PEG-G-CSFs assessed both *in vivo* and *in vitro* and related to the amount of PEG added per molecule. Many of the candidates comprised defined blends of non-PEGylated, mono-, di-, tri- and tetraPEGylated G-CSFs. The amount of PEG per molecule represents the product of molecular weight of the PEG moiety and the average number of additions per molecule. → – indicates the data obtained with the final pegfilgrastim selected for further development. △ – the proliferation of 32D clone 3 cells (a murine G-CSF dependent cell line) as measured by reduction of Alomar blue. □ – weighted AUC was obtained from daily average ANC from mice (5 per timepoint) weighted by multiplying by the number of days after injection, then summed. This weights selection in favor of longer acting forms.

Targeting the N-terminal residue of proteins is an attractive option, offering the benefits of a single, defined site, a known relationship to the receptor binding domain of the cytokine and relative simplicity in the required chemistry. Several methods have been developed to target the N-terminus for PEGylation including chemical activation and enzyme ligation; however, few have been exploited to develop viable product entities. The method employed for the fabrication of pegfilgrastim was based on a reductive alkylation process to direct conjugation of PEG to the N-terminal methionine of filgrastim (r-metHuG-CSF). This was achieved by taking advantage of the different pKa of the α-amino group of the N-terminal methionine (pKa 7.6–8.0) in contrast to the ε-amino group (pKa 10–10.2 [55]) found on the lysines throughout the molecule.

Mono-N-terminal poly(ethylene glycol) conjugates of filgrastim

Numerous PEG-G-CSF conjugates were prepared for activity screening. Linear mono-functional monomethoxy PEG aldehydes of various molecular

weights (between 12 and 30 kD) were used to prepare derivatives. More complex, branched PEG forms were also assessed. The method included stirring a cold buffered (pH 5) solution of rmetHuG-CSF in the presence of a five fold molar excess of mPEG aldehyde in 20 mM sodium cyanoborohydride. The degree of PEGlyation was tracked with HPLC until after around 10 h 92% of the protein was shown to be mono-PEG conjugate. The site of PEG attachment was determined by endoproteinase mapping and confirmed to be single site of PEG conjugation at the N-terminus of the protein [55, 56].

Preclinical and clinical development of Pegfilgrastim

Screening activity

The screening process for PEGylated derivatives was designed to select a candidate with prolonged action *in vivo* and retention of the maximum *in vitro* activity. As mentioned above, the engineering of darbepoetin is in some ways analogous to the development of pegfilgrastim and some lessons from that program are salutory. The literature published on the development of darbepoetin illustrated that the derivatives that were most potent *in vivo* were among the least active *in vitro*. In the case of erythropoietin analogs the form with the highest affinity for the receptor and the highest *in vitro* activity (a deglycosylated form) paradoxically had no detectable activity *in vivo*; presumably because it was cleared from the body in a matter of only minutes. At the other extreme, highly modified forms (with high sialic acid content) had lower affinity for the receptor, were also several fold less active *in vitro* yet were the most spectacularly effective when injected *in vivo*. This led us to conclude that in assessing the activity of derivitized cytokines, assays carried out *in vitro* where affinity is a dominant determinant, may be misleading in candidate selection. The aim in developing a pharmacokinetically advantaged derivative is not to increase affinity (indeed the opposite would appear to be true) but to obtain the optimum blend between longevity and the [likely?] reduction in affinity detected by somewhat artificial *in vitro* assays. On a practical level this meant that though assessment of the *in vitro* activity of various PEGylated G-CSFs was performed, it was considered with little weight against the *in vivo* assessments of activity.

A relationship was defined between the molecular weight of PEG added to various PEG-G-CSF derivatives and the performance of the conjugates both *in vitro* and *in vivo*. There existed weak relationships between average MW of added PEG and activity *in vivo* (a positive relationship) and *in vitro* (an inverse relationship) – see Figure 2. The final selection was made based upon several parameters including retention of around 70% of the *in vitro* activity of the parent molecule in combination with substantial improvement in weighted (in favor of longer acting forms) AUC of ANC response. Other *in vivo* parameters

were also considered as part of the selection process – mobilization of PBPC and reversal of 5-fluorouracil induced myelosuppression in mice in addition to factors such as consistency/robustness of the production process, availability of raw materials and formulation properties.

Having made the selection based on data from mice that indicated a prolonged mode of action, several pieces of information were collected to assess whether other design parameters had been met. In a study in groups of normal or bilaterally nephrectomized rats, an intravenous dose of pegfilgrastim was

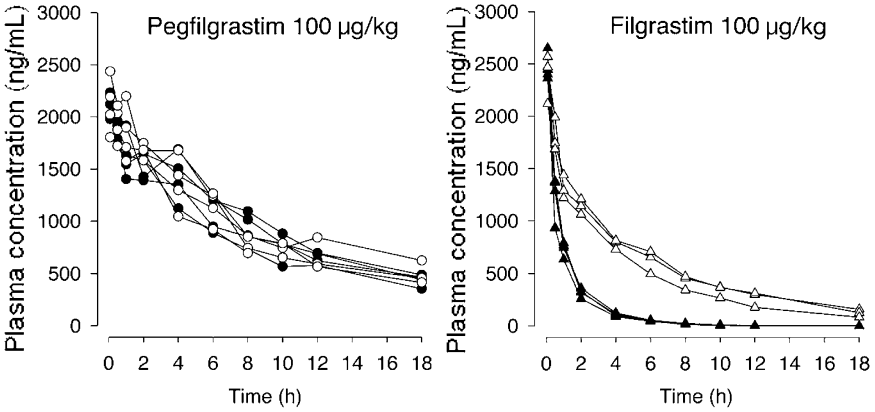

Figure 3. Pegfilgrastim clearance from the plasma of treated rats is independent of renal function. Plasma levels after a single intravenous injection of 100 µg/kg of pegfilgrastim (left) and filgrastim (right) in normal (closed symbols) or bilaterally nephrectomized (open symbols) rats (n = 3 or 4, individual data shown). Note on the left the presence or absence of kidneys makes little difference to the clearance of pegfilgrastim, but the clearance of filgrastim (right) is significantly affected by the existence of a functional kidney. Adapted from Yang et al. [61].

cleared with identical kinetics in both groups (see Fig. 3). Filgrastim, in contrast, was eliminated much more rapidly in normal animals than in those lacking kidney function. This suggested that the new form was resistant to renal clearance as was hoped from the design process. The second feature of the molecule that was considered essential in the design stage was that it should remain sensitive to neutrophil-mediated destruction. Figure 4 illustrates that both filgrastim and pegfilgrastim are removed from culture supernatent by neutrophils isolated from the blood of normal volunteers. Pegfilgrastim is relatively protected, but since both PEG- and non-PEG-G-CSF were removed both could possibly be cleared from the body by this process.

The stage was therefore set to initiate more advanced testing. Toxicology studies had revealed no new safety concerns – the only observations made were associated with exaggerated pharmacology, as would be expected with a more active derivative of G-CSF.

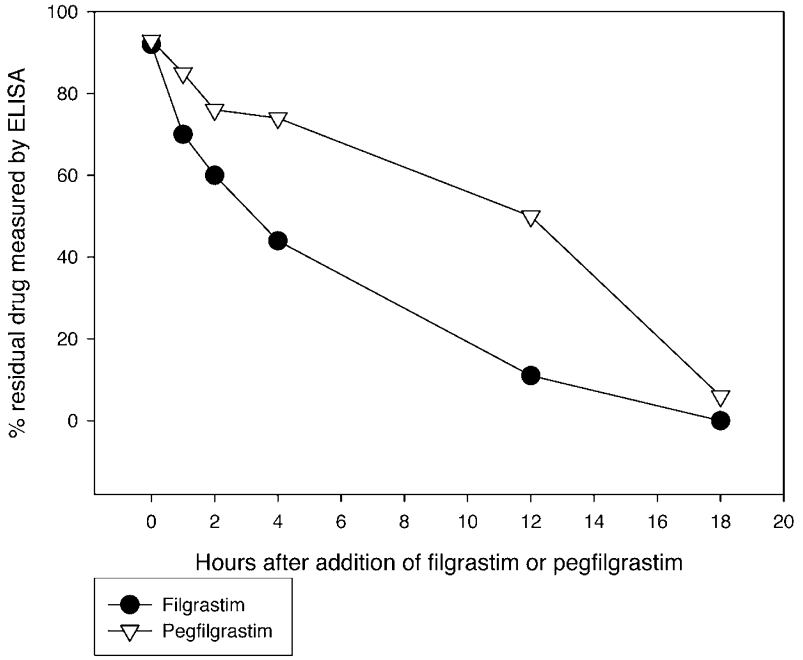

Figure 4. The ability of normal human neutrophils to remove filgrastim and pegfilgrastim from culture supernatent. Adapted from Briddell et al. [30].

Clinical development

Daily dosing with filgrastim is required for clinical efficacy and experiments in animals have illustrated that no matter how far the dose of filgrastim is escalated, the requirement for frequent administration cannot be avoided [14, 57].

Among the early clinical experiments was a simple dose escalation study in normal volunteers (see Fig. 5). Neutrophil counts increased in a dose dependent manner. Other Phase I trials were uneventful and a Phase II trial in patients with non small-cell lung cancer [58] confirmed that many of the initial design objectives had been fulfilled for pegfilgrastim, including an extended duration of action. This study employed an interesting cycle 0/cycle 1 design in which patients intended for treatment received pegfilgrastim prior to chemotherapy (cycle zero) then again immediately after chemotherapy (cycle 1). This allowed each patient to act as their own control and made possible analysis of the effects of chemotherapy induced neutropenia on pegfilgrastim. In cycle zero there was a dose dependent neutrophilia not dissimilar to the data reported from the earlier Phase I trial. The chemotherapy, as expected, caused a significant neutropenia in cycle 1 (Fig. 6A), but the critical analysis from this paper, from a mechanism perspective, is the variation in pharmacokinetics from cycle 0 to cycle 1 (Fig. 6B). Peak serum levels attained in response to

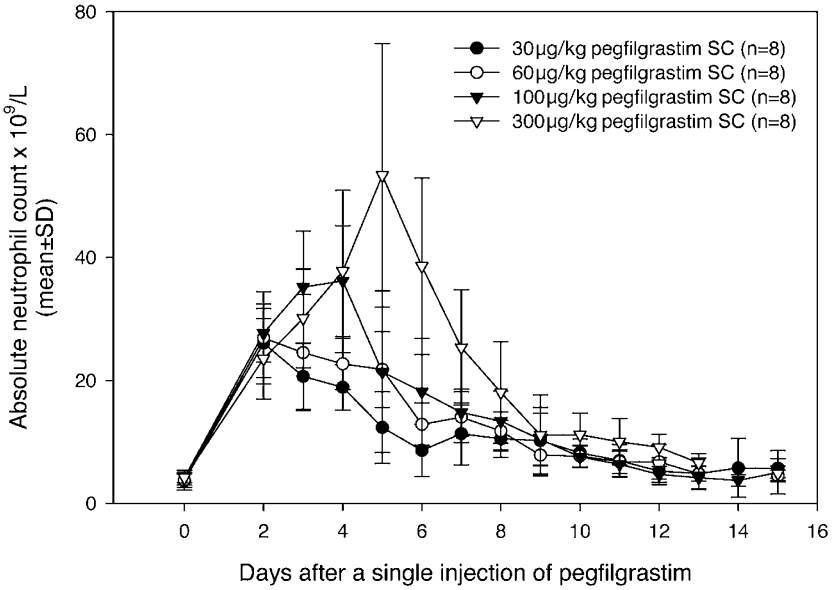

Figure 5. Neutrophil response in normal volunteers injection subcutaneously with a single escalating dose of pegfilgrastim (adapted from Molineux et al. [57]).

100 µg/kg either before or after chemotherapy were similar – at around 100 ng/mL compared with 10–20 ng/mL peak levels in the group receiving the recommended dose of filgrastim. Obviously, the pegfilgrastim recipients received 20 times more drug that accounts for the higher maximum concentration attained, though the rate of loss, once underway, was broadly comparable between pegfilgrastim and filgrastim. The main difference between cycle 0 and cycle 1 was the time at which that clearance began. For several days post chemotherapy no pegfilgrastim is lost from the serum–serum concentration remained constant for several days – a phenomenon that had not been seen in the pre-chemotherapy cycle. However, starting around nine days after chemotherapy pegfilgrastim was lost from the serum – and lost at a precipitous rate. This turning point coincides with the recovery of neutrophils after chemotherapy. This observation is compatible with the concept of self-regulation where pegfilgrastim levels remain broadly stable accelerating neutrophil recovery and when that neutrophil recovery begins the new neutrophils then clear the drug.

Various Phase II trials uncovered no untoward activities of pegfilgrastim and two randomized double blind Phase III trials were initiated with slightly different designs. Both were conducted in breast cancer patients receiving doxorubicin and docetaxel chemotherapy but in one trial later to be reported by Green et al. [59] patients received pegfilgrastim at a fixed dose of 6 mg irrespective of body weight, the complementary trial reported by Holmes et al. [60]

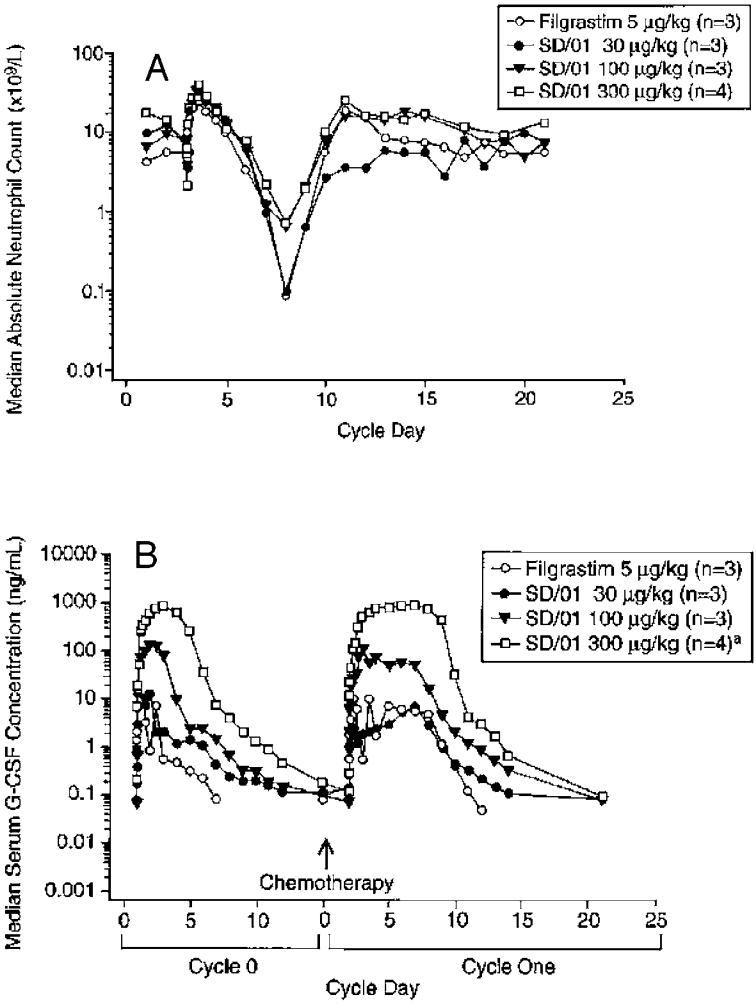

Figure 6. Phase II data with pegfilgrastim (SD/01) in lung cancer patients treated with escalating doses in cycle 0 (pre-chemotherapy) or cycle 1 (post-chemotherapy). Panel A. Neutrophil counts after chemotherapy in cycle 1. Panel B. Pharmacokinetics of pegfilgrastim (SD/01) in cycle 0 (pre-chemotherapy, normal ANC) and in cycle 1 (post-chemotherapy and neutropenic – see Panel A). Note the prolonged exposure in cycle 1 *versus* cycle 0 and the precipitous clearance of SD/01 in parallel with neutrophil recovery. Johnston et al. [58].

used conventional dosing by body weight at 100 µg/kg. The somewhat unusual step of using a fixed dose of a biological was taken based upon analysis of the various Phase II trials in terms of the total dose received by individual patients (of body weights ranging from 46–125 kg) and the duration of their severe neutropenia (see Tab. 2). It is apparent that irrespective of body weight, the days of severe neutropenia (DSN) were similar, perhaps even shorter at the higher body weights. Both Phase III trials focused on DSN as the primary end-

Table 2. The duration of severe neutropenia in the 2 phase 3 trials of pegfilgrastim in which the drug was dosed based upon patient body weight or administered as a single fixed dose.

		Days of severe neutropenia			
		Cycle 1	Cycle 2	Cycle 3	Cycle 4
Per weight dosing (100μg/kg)[59]	pegfilgrastim	1.1	0–1 (in 98% of patients)		
	filgrastim	1.6	0–1 (in 96% of patients)		
Fixed dose (6mg)[60]	pegfilgrastim	1.8	1.1	1.1	1.0
	filgrastim	1.6	0.9	0.9	1.0

point and in both cases DSN was shown to be non-inferior to filgrastim (an unusual endpoint useful in making a statistical comparison to an active control). In the trial where patients received a 6 mg fixed dose, the 77 patients who received pegfilgrastim and the 75 who received filgrastim had 1.8 and 1.6 days of severe neutropenia, and in the by-weight trial 1.1 and 1.6 respectively.

The first warning sign that a neutropenic cancer patient may be developing an infection is becoming febrile (having an elevated temperature). Febrile neutropenia is defined as a temperature of greater than 38.2 °C when accompanied by neutropenia and often prompts the use of anti-infectives even though in many cases an infection cannot be confirmed. Combined data from both the by-weight and fixed dose trials showed that pegfilgrastim reduced significantly the occurrence of febrile neutropenia even compared to filgrastim (11% *versus* 19% – no placebo control group was reported). It is unknown to date why this may be the case. It is tempting to speculate that the front-loading, high dose, or lack of daily fluctuations in drug or ANC levels in the pegfilgrastim recipients may play a role, but dissecting out each of these components has not proven feasible to date.

Bone pain, which is the major side-effect reported for filgrastim, remained the only significant pegfilgrastim-related event that could be teased out of the complex symptoms reported by cancer patients undergoing chemotherapy.

Conclusions

Pegfilgrastim is a rationally designed cytokine derivative engineered specifically to enhance its properties as a therapeutic. The design evolved from understanding the limitations placed on the parent drug by its brief residence time in the body. Of the two routes of filgrastim clearance that contribute to its rapid loss, one – the neutrophil-mediated pathway, is related to the product of the drug's effects, the other is a relentless, linear process based on loss through the kidney. Pegfilgrastim, for the first time, separated these effects and

removed renal loss as a significant phenomenon. This left neutrophil-mediated destruction as the only significant route of drug elimination. Since stimulation of neutrophil production is the reason why G-CSF is administered to patients, this novel drug eliminates the requirement for dosing based on the patient's individual characteristics or response.

Though pegfilgrastim is the latest of a new generation of 'designer cytokines' it is unlikely to be the last. The evolution of protein therapeutics from natural materials purified from animal or human sources, through the fabrication of recombinant equivalents to semi-synthetic hybrid molecules (like pegfilgrastim) and eventually to fully synthetic drugs will continue to optimize the utility of this class of drugs improving patient convenience, compliance and response rates but hopefully retaining the exquisite specificity and side effect profile of the parent hormones.

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