ANGIOGENIC EFFECTS OF PEGFILGRASTIM; A HALLMARK IN THE FIELD OF NANO CHEOTHERAPEUTIC

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Abstract

The technology of nano drugs has brought a big revolution in the field of therapeutics and has given a better approach to deal with oncogenic proteins and destructing them by site specific payload drug. Now it is easy to treat small cancerous molecules which are lethal due to their capability of potential growth and detrimental effects on human health. Nano technology and nano particle drug delivery system have a better approach over the conventional treatment approaches. Huge ranges of different nano particles have different properties so that we can choose them according to our interest. Pegylated filgrastim have better effects as compared to filgrastim in neutropenia and also causes increase in retention time of drug. In this review we have tried to discuss every aspect of pegfilgrastim covering its synthesis, pharmacokinetics and pharmacodynamics. More supplementary work is needed for better identification of side effects as well as adverse effects of pegfilgrastim.

NANO PARTICLE/ NANO TECHNOLOGY

Nano technology shells a number of aspects of science referred to as biology, engineering, chemistry and medicines. The formal name converts the dimension to the minimal quantity of the drug that prevails itself in nanoparticles for its safe and efficient delivery to the target site, and increasing the bioavailability to prevent itself from degradation and interaction. Nanoparticles are novel in nature. Their novelty and efficacy made them superior over conventional drug delivery system (**as shown**

in Fig. 1). Numerous types of nanoparticles used in drug delivery system are chosen due to their structure, ligand and target site. The nanoparticles under taken for drug deliveries are liposomes, nano capsules, nano emulsions, solid lipid nanoparticles and polymeric nanoparticles [1-3]. Recent years have improved the importance of these particles for their utilization in drug targeting, as a therapeutic agent and also in imaging and controlled drug release [4, 5].

INTRODUCTION

NANOPARTICLE AND DRUG DELIVERY

Drug delivery and related pharmaceutical preparations in the category of nano medicine should be viewed as a science of Nano scale system (10-100nm), consisting a major pharmaceutically active part which have to be delivered to the site of infection, although Nano particle formulation of the drug itself is also possible. The whole system leads to perform unique function related to treating, preventing and diagnosing the diseases called smart drug [6, 7]. The basic goals for research in nanotechnology in drug delivery include

1. Toxicity reduction besides maintaining therapeutic effect
2. Specific targeting of drug and its delivery
3. Biocompatibility and safety
4. Development of newer and safer medicine

The main issue in research is to find specific carrier drug delivery system, which fulfills the following basic parameters for designing of nano materials, and these are;

a) Drug incorporation and its release,
b) Stability and its shelf life,
c) Biocompatibility,
d) Distribution and targeting,
e) Functionality.

In addition, the carriers should be observed for its possible adverse effects and its biodegradation after releasing the payload drug.

Table S1 represents some of the chemical structures used for the pharmaceutical carriers. Precisely none of them fulfill all the parameters stated above.

The aim of nanoparticle using in drug delivery is to enhance delivery to target cell, or uptake by target cell and reduction in toxicity of the free none target organ. Both

results in increase of therapeutic index. For this aim long circulation time and specific targeting nanoparticles are needed. **Some chemicals are given in table 1**.

Most compounds are biodegradable polymers after release of payload drug. Nano-particles release the drug upon its degradation. One problem in specific drug carrier nanoparticle is their clearance from mononuclear phagocytic system, in liver and spleen and this problem is overcome by surface modification of particular nanoparticle by coating with poly ethylene glycol (PEG), resulting in increased time in shelf life and in circulation inhibiting reorganization by mononuclear phagocytic system. Surface coating is also shown to be necessary for preventing the agglomeration of the particle. Many types of coatings are used for preventing agglomeration and keep them in suspension form with in the colloidal solution. Several coatings are used for this goal are, poly ethylene glycol (PEG), poly vinyl pyrrolidone (PVP) and in natural source polymers like dextran and chitosan are used.

Nanoparticle coating regulates the nanoparticles targeting, solubility and stability. For drug application a nanoparticle should be polar to give solubility in aqueous medium and for preventing aggregation. Polyethylene glycol joined with terminal hydroxyl or methoxy group prevents it from interactions [8, 9]. Biological macromolecules linked to nanoparticles, which address the tag to target site of the body [10] and protein or RNA molecules movement to the specific cells [11, 12]. Monoclonal antibody, apatamers or peptide should covalently attach to nanoparticles in controlled number. These multivalent having many targeting groups cluster receptor gives strong anchoring in spite bearing single site [13-18]

The size of nanoparticle is the major critical parameter to be taken into consideration for its clearance from mononuclear phagocytic system and other surface phagocytic system. Size distribution may also influence the bioavailability of the active drug e.g. for liposomes if the size >100nm then the clearance rate increases with size whereas below 100nm charges play an important role. However this criteria is not same for all nanoparticles rather composition is also important. Besides physical means discussed above there are factors like light and heat, which may also affect the release of payload drug on targeted site [19, 20].

NANO PARTICLES IN CANCER

Nano particles at present have been utilizing in many treatment aspects for cancer like diagnosis, pre-detection, targeted therapy and cancer bioinformatics. In cancer the

selection of nanoparticles minimize the spectrum of particles for the effective and specific site delivery of payload drug. The aspects discussed below are of prior importance while engineering nano particles [21].

a. When particles injected they should be cleared by phagocytic system and minimize the undesirable cytotoxicity.
b. History should be safe used and biocompatibility proven.
c. Rate of polymer degradation and release of payload entity should be safe.

TYPES OF NANOPARTICLES

There are various ways by which nanoparticles are classified. One of the most basic way of their division can be favored in a book written by chemists Mark and Daniel Ratner [22], is under taken.

A. Polymeric Nanoparticle

Polymeric nanoparticles (PNPs) consist of a decomposable polymer. Biocompatibility is a crucial feature for potential application as tissue engineering, drug and gene transport and new vaccination strategies. Most biodegradable polymers entails of synthetic polyesters like polycyanoacrylate or poly(D, L-lactide) and allied polymers like poly(lactid acid) PLA or poly(lactide-co-glycolide) to give a better approach. Latest developments also comprise natural polymers like chitosan, gelatin, and sodium alginate to overawe some toxicological problems with the synthetic polymers. Polymeric nanoparticles characterize a significant improvement above traditional oral and intravenous (IV) approaches of administration in terms of efficiency and effectiveness. Polymeric nanoparticles may have engineered specificity, permitting them to deliver a superior concentration of pharmaceutical agent to a desired location [23, 24]. Polymeric materials are used to make up for these particles, which have small cavities where a drug is stored. Once enter into the GIT of the patient, the enclosed drugs are released (**as shown in Fig. 2**). This method facilitates a continuously smooth delivery over an extended period of time [25, 26].

B. Nonpolar Nano-Coating

Many drugs cannot pass through membrane cells due to their polarity. Polar drugs have difficulty reacting with nonpolar membrane cells. To overcome this problem, non-polar nanoscale coatings can be devised to encapsulate and deliver the polar drug with no problem [25, 27].

C. Dendrimers

Dendrimers are extremely diverged and symmetrical structures between approximately 1 and 10nm [21, 28, 29]. These are an exclusive class of polymers highly diverged macromolecules whose size and shape can be quite controlled. Dendrimers stay fabricated from monomers using either convergent step growth polymerization. Symbols of poly amido amine based dendrimers are the well-defined structure; mono-dispersity of size, surface functionalization aptitude, and stability is property of dendrimers that mark them attractive drug carrier candidates. Drug molecules can be incorporated into dendrimers by encapsulation. Dendrimers are being explored for both drug and gene delivery (**as shown in Fig. S1)**. Dendrimers used in drug delivery studies habitually incorporate one or more of the given polymers polyamidoamine (PAMAM), melamine, poly (L-glutamic acid) (PG), polyethyleneimine (PEI), poly (propyleneimine), and poly (ethylene glycol) (PEG) [30-32]

D. Smart Materials and Nanoparticles

The idea for the development of these types of materials is to make them react only under certain specific conditions. For example, polymer nanoparticles are equipped with bio receptors in their outer shells, which can detect and respond to specific biochemical signals that will release the drug from inside of the particle [33, 34].

E. Magnetic Nanoparticles

These are bound by molecular recognition of the specific cells, which will favor the drug to deliver (**as shown in Fig. S2)**. An external magnetic field can manipulate the location of the nano dot therefore it controls the delivery of the drug [25, 35, 36].

F. Liposomes

Liposomes are spherical particles comprised of one or more lipid or phospholipid bilayers and contain a placing between the two layers. A benefit of liposomes is that the structure can contain lipophilic as well as hydrophobic, and amphiphilic molecules more compounds with different solubility's can be incorporated in the arrangement [37-40]. These liposomes are shaped due to unfavourable interactions of the phospholipids with the aqueous medium, so that the polar head-groups position towards the media, whereas the hydrophobic carbon tails are forced to cluster into a two layers [41]. Liposomes can be categorized according to their size and the number of bilayers [42, 43]. A great advantage of bilayers in manufacturing is the low CMC (Critical Micellization Concentration) at which they form compared to micelles. Typically, the CMC of liposomes are an order of four to five lower than those of micelles [44]. Frequently used Phospholipids to form the bilayer are phosphatidylcholine (charge neutral), and the negatively charged phosphatidic acid, phosphatidyl glycerol, phosphatidylserine, and phosphatidyl ethanolamine (**as shown in Fig. 3**). Archaesomes are somewhat more thermo stable and resistant to oxidation, chemical and enzymatic hydrolysis when compared with liposomes. They are also more resistant to low pH and bile salts that would be encountered in the gastrointestinal tract (GIT) [45]. This makes them ideal aspirants to protect anti-oxidants during food processing. Now it has become feasible to use liposomes to deliver functional components such as nutraceuticals, antimicrobials, and flavours to foods [46-53].

Relative Sizes of General Particles

Different size of relative nanoparticles are produced **(as shown in Fig. S3).**

SYNTHESIS OF NANO PARTICLES

For the production of nano drugs, synthesis of nanoparticles is the core attempt to deal with and for attaining these goals, number of strategies is used. There are two main classes for the synthesis of nanoparticles; attrition and pyrolysis [54-56]. Attrition helps by making particles through ground ball mill, and planetary ball mill or by other size reducing mechanisms top down method (mechanical), bottom up technique (wet chemistry). Top down method reduces the size of particles by ball milling or by grinding. While in Bottom up technique formation of the nanoparticles starting from ions or molecules and make the larger structure (**as shown in Fig. S4)**. Pyrolysis

attains these goals through different techniques. Form in place vacuumed coating and spray coating is used to incorporate lithography. While in gas phase synthesis inert gases are used to synthesize nanoparticles from metals with low melting points and further include laser ablation, plasma vaporization, and vapor synthesis [57-60].

A. Mechanical Methods

1. Sol-Gel Method

The sol-gel process is a technique of chemical solution deposition widely used recently in the areas of materials science and ceramic engineering [61-63]. Such approaches are used predominantly for the fabrication of materials (typically a metal oxide) starting from a chemical solution (*sol*, short for solution) which acts as the originator for an unified network (or *gel*) of either distinct particles or network polymers [64]. Specific precursors are metal alkoxides and metal chlorides, which undergo hydrolysis and polycondensation reactions to form either a network "elastic solid" or a colloidal suspension (or dispersion). A system composed of distinct (often amorphous) sub-micrometer particles dispersed to assorted degrees in a host fluid. Formation of a metal oxide engages connecting the metal centers with oxo (M-O-M) or hydroxo (M-OH-M) ties, consequently generating metal-oxo or metal-hydroxo polymers in solution. Thus, the solution evolves towards the formation of a gel-like diphasic system containing both a liquid phase and solid phase (**as shown in Fig. S5)**. Morphologies range from discrete particles to continuous polymer networks [65].

In the paradigm of the colloid, the volume portion of particles (or particle density) may be so low that a suggestive amount of fluid may need to be removed initially for the gel-like properties to be predictable. This can be capable in any number of ways. The simplest method is to allow time for sedimentation to appear, and then transfer off the remaining liquid. Centrifugation can also be used to accelerate the process of phase separation.

Removal of the residual liquid (solvent) phase requires a drying process, which is typically complemented by a significant amount of shrinkage and densification. The vital microstructure of the final component will clearly be strongly influenced by changes employed during this phase of processing. After that, a thermal treatment, or firing process, is often necessary in order to favor further poly-condensation and enhance mechanical properties and structural stability through final sintering,

densification and gain growth. One of the distinct advantages of using this methodology as opposed to the more traditional processing techniques is that densification is often achieved at a much lower temperature.

The precursor sol can be either deposited on a substrate to form a film (e.g. by dip-coating or spin-coating), cast into a suitable container with the desired shape (e.g. to obtain a monolithic ceramics, glasses, fibers, membranes, aerogels), or used to synthesize powders (e.g. micro-spheres, nano-spheres). The sol-gel approach is a cheap and low-temperature technique that allows for the fine switch of the product's chemical composition. Even small quantities of dopants, such as organic dyes and rare earth metals, can be introduced in the sol and end up uniformly dispersed in the final product. It can be used in ceramics processing and manufacturing as an investment casting material, or as a means of producing very thin films of metal oxides for various purposes. Sol-gel derived materials have diverse applications in optics, electronics, energy, space, biosensors, medicine (e.g. controlled drug release) and separation [66-69].

2. Pyrolysis

Spray pyrolysis is a liquid-based gas phase tactic and electrospray is proficient of generating reasonable droplets as well as nanoparticles. We report a narrative synthesis route by spray pyrolysis (salt- assisted spray pyrolysis) (SASP) for the continuous synthesis of nanoparticles with variable sizes, a narrow size allocation, high crystallinity, and good stoichiometry [70, 71]. In this approach, many kinds of single crystalline nanoparticles (oxide; CeO^2, NiO, ZnO, Ba-Sr-Ti-O, Mn doped ZnS, metal; Ag-Pd) can be prepared. This route can compromise good controllability of particle size, chemical alignment, and material crystallinity [72-74].

In pyrolysis, a vaporous precursor (liquid or gas) is forced through an outlet at high pressure and scorched. The subsequent solid (a version of soot) is air organized to recuperate oxide particles from by-product gases. Pyrolysis often results in sums and agglomerates rather than single primary particles (**as shown in Fig S6)**.

B. Synthesis of Nanoparticles by Chemical Methods

1. Combustion: Combustion of iron penta carbonyl and carbon monoxide is one method of producing nanoparticles. In this procedure a chain of iron oxide aggregate is produced [75-77].

2. Fe_3O_4 Nanoparticles: Fe_3O_4 nanoparticles can be produced from a solution of ferric chloride ($FeCl_3$) and ferric sulfate ($FeSO_4$) under a magnetic influence. The mixture is prepared and kept under argon protection however an ammonia aqueous solution is quickly mixed into the mixture. The resultant is kept under a circular magnet, and the magnetic field differs according to the location of the flask relative to the magnet. After aging for one day, the precipitate is filtered and washed with deionized water and ethyl alcohol and the Fe_3O_4 nanoparticles were produced [78-82].

3. **Co-Precipitation:** In co-precipitation two methods are used to produce magnetic nanoparticles.
   i. Ferrous hydroxide suspensions can be partially oxidized with different oxidizing agents. This method produces nano-spheres with diameters of 30 to 100 nm [83, 84].
   ii. The other method ages a mixture of ferrous and ferric hydroxides in aqueous medium. In this method, adjusting the pH or ionization of the medium can control the particle size. Size ranges from 2-15 nm.

4. Micro-Emulsion: Micro-emulsion uses a mixture of water and oil to create micro cavities that can control particle nucleation and growth [84-86].

5. Polyols: In Polyols a solution with dissolved metallic salts can cause metal precipitation of fine metallic nanoparticles [84, 87, 88].

6. High Temperature Decomposition of Organic Precursors: In this method iron precursors decompose in the presence of hot organic surfactants. It allows a good control of size and crystallinity of individual magnetic iron oxide nanoparticles [84].

NEULASTA

It is a drug with generic name of pegfilgrastim. Filgrastim is a protein soluble in water consists of 175 amino acids and weight almost 19 kilo Dalton. Fermentation is the

mother source for obtaining filgrastim. Genetically altered plasmid containing human G-CSF gene is transformed in E-coli in which it is propagated. Pegfilgrastim is a conjugate of methionyl-humen G-CSF filgrastim and mono-methoxy polyethylene glycol. Pegfilgrastim of weight 20 KD mono-methoxy polyethylene glycol is attached at N-termin-as-methionyl of filgrastim. This pegelated filgrastim (pegfilgrastim) permits active substance to stay longer rather eliminates early [89-91].

Pharmacology

G-CSF is an endogenously produced colony-stimulating factor released by endothelial cells and monocytes fibroblast. It stimulates the synthesis of neutrophil land its proliferation, differentiation and end cell activation functionally. Exogenous administration of the colony stimulating factors commits the stem cell to produce white blood cells for fitting. Cancerous patient are given these factors due to the eventually decrease in WBCs because of chemotherapy and radiations and efficiently effects by reducing neutrophenic phase (time in which people are susceptible to infection) [92-94]. Pegfilgrastim is a colony-stimulating factor. These are glycoproteins that act on hematopoietic stem cells by binding to the cell surface receptors.

PHARMACOKINETICS

Neulasta Dosage

Neulasta recommended dosage for prevention of infection under taking chemotherapy is 6 mg injection just under skin once per chemotherapy cycle. Suggested dosage is same for all adults, besides of age, kidney and liver function. Dose should not be taken before 24 hours after chemotherapy and is usually given the day after chemotherapy (more than 24 hours) [95-97].

Neulasta Solubility and Stability

Shelf life of neulasta is 3 years. It should be stored at 2-8 C^0. Neulasta may be exposed to room temperature but not more than 30 C^0, but if left at room temperature for above then 72 hours should be discorded. Do not freeze, if frozen then use only once but not use neulasta frozen two times. Do not shake before using. Excessive shaking may aggregate pegfilgrastim making it inactive. Before use neulasta should

be inspected visually, only a solution that is colorless and clear should be used [98-100].

Elimination

Half-life of neulasta is 15 to 80 hours. Serum clearance is directly related to the neutrophil count [101-104].

Renal Function Impairment

No difference in pharmacokinetics based on renal function impairment, disease even may be at end-stage [105, 106].

Impairment Hepatic Function

Have no impairment on pharmacokinetics [107, 108].

Gender

No gender-based difference in pharmacokinetics of neulasta is reported [109, 110].

PHARMECODYNAMICS

Side Effect of Neulasta

General side effect of pegfilgrastim linked to chemotherapy when administered show these effects e.g. nausea, skeletal pain, fatigue, alopecia, headache, taste perversion, constipation, fever, anorexia, headache, dyspepsia, myalgia, insomnia, abdominal pain, arthralgia, generalized weakness, peripheral edema, dizziness, granulo cytopenia, stomatitis, mucositis, and neutropenic fever. Investigators suggest that these effects are the underlying cause of chemotherapy administration.

Besides other chemotherapy medicine side effect of Neulasta are rare but are strongly serious and may require immediate attention. Major side effects include signs of ruptured or enlarged spleen, breathing problems and increased growth of cancer. Taking Neulasta not all patients experience the side effects, infect many tolerate them and occur rarely [110-114].

Indication and usage

Neulasta is used patient taking myelo-suppressive anti-cancer drugs associated with febrile neutropenia and to decrease the intensity of infection [115, 116].

Effect on Bone

When Neulasta is given bone pain is reported in 57 percent of patients,
And joint pain in 16 percent of patients [110, 117, 118].

Effect on Muscle

Muscle pain in 21 percent of patients is observed taking Neulasta [114, 119, 120].

Effect on GIT

When Neulasta is given vomiting in 13 percent of patients is observed while,
Constipation in 10 percent and a few claims diarrhea and fever [121-124].

Effect on Skin

Hair loss is majorly due to chemotherapy medicine in Neulasta case it contribute a little [91, 125, 126].

Adverse Effects

Effects on CNS

When Neulasta show its adverse effect the central nervous system undergo these problems e.g. Pyrexia, headache, dizziness, fatigue, generalized weakness, insomnia, taste perversion, asthenia [127-129].

Effects on GIT

Diarrhea, abdominal pain, anorexia, constipation, dyspepsia, mucositis, nausea, stomatitis, vomiting are the conditions mostly acquired in the patients when drug is adversified [104, 130, 131].

Hematologic-Lymphatic

Granulo-cytopenia, neutropenic fever, leukocytosis, sickle cell crisis [132-134].

Musculoskeletal

Bone pain, myalgia, arthralgia, and skeletal pain [135, 136].

Local

Injection site reactions including erythema, induration, and pain [137, 138].

Hypersensitivity

Allergic reactions including anaphylaxis, skin rashes and urticaria [139].

Drug Interaction

When Neulasta is taken with chemotherapy medication drug interaction may occur. Neulasta actually increase the low white blood cells decreased by chemotherapy medication. When Neulasta is taken with Lithium interaction may occur that can lead to serious problems such as heart attack or strokes. Pegfilgrastim can interact with other medicines may lead to Neulasta drug interaction include; medications used for chemotherapy e.g. Lithium, estalith, lithobid. Lithium and Neulasta both increase the production of white blood cells. As thinking to be beneficial it could be dangerous by increasing the count too high and lead to serious problems as stroke and heart attack [140-143].

Phase II Study of Pegfilgrastim

Pegfilgrastim comprises filgrastim bound to a 20-kDa polyethylene glycol molecule. Pegylation decreases renal clearance and prolongs circulation half-life, stemming in

sustained drug pursuit [144-147]. Pegfilgrastim clearance is predominantly mediated by neutrophils, so the drug concentration is sustained in the circulation during chemotherapy induced neutropenia and rapidly eliminated on neutrophil recovery [148-150]. In adults single dose of pegfilgrastim (either 100 g/kg or 6 mg) after chemotherapy has demonstrated safety and efficacy similar to daily filgrastim [151-153]. The recommended adult dosage is 6 mg administered subcutaneously once per chemotherapy cycle. A limited number of studies have evaluated pegfilgrastim dosing in pediatric patients [154, 155]. This phase II study was designed to gauge the safety, clinical response, and pharmacokinetics of pegfilgrastim in pediatric patients with sarcoma receiving dose-intensive chemotherapy. Patients randomly assigned to the filgrastim group received 5 g/kg filgrastim subcutaneously daily beginning approximately 24 hours after chemotherapy completion in week 1 (generally, day 4 in cycles 1 to 3 and day 6 in cycles 2 and 4) and continuing either until a post-nadir Absolute Neutrophil Count (ANC) of 10 x 109/L was achieved or until 24 hours before the next scheduled chemotherapy cycle, whichever occurred first. Patients randomly allocated to pegfilgrastim received 100 g/kg subcutaneously approximately 24 hours after chemotherapy completion. Dose escalation of pegfilgrastim was to be considered for an age stratum if more than one of the six patients failed to achieve ANC recovery (ANC 0.5 109/L) in cycle 1. An age stratum was closed to accrual after two successive groups of six patients (within the age stratum) achieved ANC recovery. Thus, minimum of 42 patients (12 pegfilgrastim and two filgrastim in each of the three age strata) were required to complete study-specified enrollment.

Study indicates that pegfilgrastim 100 g/kg administered once per chemotherapy cycle has analogous efficacy and safety to filgrastim at 5 g/kg dispensed daily in pediatric patients receiving chemotherapy. Median duration of rank 4 neutropenia was 5.0 days in the pegfilgrastim group and 6.0 days in the filgrastim group in cycle 1 and 7.0 days for both groups in cycle 3. Over the course of the study, FN occurred in similar proportions of patients in the two treatment groups (68% pegfilgrastim; 83% filgrastim). Two studies 11, 25 that used a similar dose-intensive regimen of VDC/IE in pediatric patients reported a median duration of grade 4 neutropenia of 5.0 days for pegfilgrastim and 6.0 days for filgrastim, a variance that was not clinically significant. Wexler et al also reported a similar incidence of FN in 11 patients receiving pegfilgrastim and filgrastim (57% and 83%, respectively) after chemotherapy cycle 1.

Of note, study included a younger patient population (from 28 days to 21 years of age, compared with 1 to 25 years in Wexler et al and 3 to 25 years in Fox et al) [156, 157].

Pharmacokinetic profiles of pegfilgrastim and filgrastim in this study are stable with those in adults [150, 151, 158]. Whereas filgrastim is cleared by neutrophils and predominantly by the kidneys, thus having a relatively short serum half-life, pegfilgrastim is cleared almost entirely by neutrophils [144, 159]. Serum concentrations of pegfilgrastim are therefore sustained throughout the ANC nadir, with quick clearance of the drug on neutrophil recovery. Neutrophil recovery, defined as an ANC 0.5 109/L after nadir, occurred in 36 of 37 patients in cycle 1; the single patient without neutrophil recovery was in the 0-5 years age group. Median drug exposures in cycle 3 were lesser than those in cycle 1. This finding may be explained by the expansion of neutrophils and neutrophil precursor mass over time leading to quicker drug clearance, consistent with a neutrophil-mediated clearance mechanism [160]. Among patients receiving pegfilgrastim, those in the 0-5 year's age group experienced a longer interval of neutropenia than older patients. Since the youngest patient unit also had a higher average exposure to pegfilgrastim than did the other two associates, the more prolonged neutropenia experienced by the youngest children in this study is likely to be due to a greater relative exposure to myelo-suppressive chemotherapy rather than to lacking pegfilgrastim dosing. Still, carefulness should be applied regarding comparisons between age groups because of the small sample sizes.

Mode of Action (MOA)

1. GCSF Receptor, functions and mutation

G-CSF is a monomer that shares sequence homology with IL-6, is synthesized by a variety of cells, involving stromal cells, fibroblasts, and endothelial cells, usually in response to inflammatory stimuli such as lipopolysaccharide (LPS), tumor necrosis factor (TNF), and IL-1. The large ECD of its receptor contains one immunoglobulin and three fibronectin domains as well as the cytokine receptor module, and it binds G-CSF with high affinity. G-CSF is the physiological regulator of neutrophil production, stimulating the proliferation and differentiation of committed neutrophil progenitor cells without affecting other granulocytic lineages, and its levels are increased during infection. It also synergizes with IL-3 or SCF to stimulate the proliferation and

differentiation of primitive multipotent hematopoietic progenitor cells. However, mice lacking G-CSF or its receptor, while neutropenic, possess some nature neutrophils and have only a mild reduction in committed granulocyte-macrophage progenitor cells, suggesting that G-CSF is not necessary for neutrophil lineage commitment. It also enhances the survival of mature neutrophils and may prime their functional responses [161].

Granulocyte colony-stimulating factor is the major hematopoietic growth factor ruling the granulo-poiesis [162, 163]. G-CSF regulates the proliferation, differentiation, and survival of myeloid originator cells. The biological activities of G-CSF are arbitrated by a specific cell surface receptor, a single trans membrane protein that is a fellow of the cytokine receptor superfamily. Similar to superfamily members, the G-CSF receptor holds no intrinsic kinase activity in the cytoplasmic domain and transduces signals via interacting with cytoplasmic kinases. Stimulation of cells with G-CSF activate multiple signal transduction pathways such as Janus kinase/Stat [163, 164] mitogen-activated protein kinases including extracellular signal-regulated kinase (Erk) 31/2, Erk5, c- Jun N-terminal kinase, and p38 (8 –13), phosphatidylinositol 3-kinase/Akt (14, 15), and Src family kinases [165]. G-CSF receptor gene mutation has been identified in a subgroup of patients with severe congenital neutropenia (SCN) [166-169]. A myeloid disorder categorized by profound selective neutropenia and a maturation arrest of bone marrow myeloid cells at early stages of development. These mutations initiate premature stop codons in the *G-CSF receptor* gene, commanding to the carboxyl-terminal truncation of 82–98 amino acids. SCN patients with G-CSF receptor mutations are subject to acute myeloid leukemia [170]. When expressed in murine myeloid cells, the truncated G-CSF receptors from patients with SCN/acute myeloid leukemia transduced stronger proliferation signals than the wild-type receptor but, unlike the wild-type receptor, failed to induce granulocytic differentiation [171]. Mice carrying an equivalent G-CSF receptor mutation were neutropenic, while bone marrow cells from these mice were hyper proliferative in response to G-CSF [172, 173]. Administration of G-CSF *In vivo* resulted in markedly increased levels of peripheral blood neutrophils as compared with normal mice. Truncation of the carboxyl terminus of the G-CSF receptor ensued in dramatically augmented and prolonged activation of Stat-5 by G-CSF in hematopoietic cells, indicating that the carboxyl terminus of the G-CSF receptor is involved in the

negative regulation of Stat-5 activation [174]. Pre-treatment of cells airing the wild type G-CSF receptor with tyrosine phosphatase inhibitor vanadate also directed to increased and prolonged activation of Stat-5 by G-CSF (**as shown in Fig S7)**. Fascinatingly, vanadate had no effect on G-CSF-induced Stat-5 activation in cells expressing a truncated receptor lacking the carboxyl-terminal 98 amino acids (F. Dong). These results propose that a phosphatase or phosphatases regulated by the carboxyl terminus of the G-CSF receptor play vital role in down-regulation of G-CSF-stimulated Stat-5 activation in hematopoietic cells.

Normal Pathway of Neutrophil Production; Hematopoietic Lineage Enhanced by Neulasta.

Normal Pathway of Neutrophil Production; Hematopoietic Lineage Enhanced by Neulasta is shown in Fig. S8.

2. Action of Neulasta on GCSFR and Hematopoietic Cells

Granulocyte-Colony Stimulating Factor (G-CSF) is a growth factor and an essential cytokine belonging to the CSF family of hormone-like glycoproteins. Numerous cell types including immune and endothelial cells produce it. G-CSF binding to its receptor G-CSF-R which belongs to the cytokine receptor type I family depends on the interaction of alpha-helical motifs of the former and two fibronectin type III as well as an immunoglobulin-like domain of the latter. Recent animal studies have also revealed that G-CSF activates multiple signaling pathways, such as Akt and also the Janus family kinase-2 and signal transducer and activation of transcription-3 (Jak2-STAT3) pathway, thereby promoting survival, proliferation, differentiation and mobilisation of haematopoietic stem and progenitor cells. G-CSF is a cytokine that have been demonstrated to improve cardiac function and perfusion in myocardial infarction (MI) [91, 175]. And it was initially evaluated as a stem cell mobilizer and erythropoietin as a cytoprotective agent. G-CSF prevents left ventricular remodeling after myocardial infarction by decreasing cardiomyocyte death and by increasing the number of blood vessels, suggesting the importance of direct actions of G-CSF on the myocardium rather than through mobilization and differentiation of stem cells (**as shown in Fig 4)**. Accordingly, recombinant human (rh) G-CSF has been extensively used in clinical hematology and oncology to enable bone marrow transplantation or to

treat chemotherapy-associated neutropenia. In preclinical study, G-CSF improved cardiac function and perfusion by angio-myogenesis and protection of cardiomyocytes in myocardial infarction [175-177].

3. Interaction of GCSFR with SRE, SHP2, STAT3

G-CSF receptor (G-CSFR) which leads to activation of intra- cellular signaling paths, comprising the Janus tyrosine kinase/signal transducer and activator of transcription (JAK/STAT), ras/mitogen-activated protein kinase (MAPK), phosphatidylinositol (3)-kinase (PI3K)/AKT, and protein kinase C pathways (PKC) [163, 178, 179]The nuclear targets of most of these pathways have not been interpreted. The serum response element-1 (SRE-1) between nucleotides and of the *egr-1* promoter is responsive to G-CSF in the myeloid leukemia cell line [180]. The SRE-1 contains a vital serum response element (SRE) and two neighboring ETS protein-binding sites (EBS). In electro mobility shift assays, the serum response factor (SRF) binds constitutively to the CArG box of the SRE whereas the ETS protein Fli-1 binds the 5 Ets binding site (EBS). Moreover, the SRE and EBSs of SRE-1 are each required for highest transcriptional activation of a reporter construct containing a single copy of SRE-1 in response to G-CSF. PI3K and its downstream target AKT are activated in G-CSF stimulated BAF3 cells that are firmly transfected with the G-CSF receptor, and PI3K/AKT pathway also mediates activation of SRE-binding proteins in response to G-CSF stimulation (**as shown in Fig S9)** [163].

SH2-containing phosphatase-1 (SHP-1) is an SH2 domain holding protein tyrosine phosphatase that is primarily expressed in hematopoietic cells. SHP-1 has been acknowledged as a negative regulator of signaling through a range of receptors [181]. (c-kit) [181], erythropoietin receptor [162]. IL-3R [163], IFN-receptor Avalos [182], B cell Ag receptor [166], T cell Ag receptor [183], killer cell inhibitor receptor [184], and CD22 [185]. Moth eaten and viable moth eaten mice express essentially no SHP-1 and mutant SHP-1 proteins with evidently compromised catalytic activity separately [186, 187], and Unveil multiple defects in hematopoiesis. The most significant hematopoietic defect is the expansion of myeloid cells. Reliable with the role of SHP-1 as a negative regulator of signal transduction, hematopoietic cells from these mice displayed enriched response to numerous growth factors and cytokines. SHP-1

particularly down-regulates G-CSF-stimulated Stat activation but not affect the activation of Erk1/2 and Akt that are stimulated by G-CSF.

Conclusion

Nano technology and Nano particle drug delivery system have a better approach over the conventional treatment approaches. Huge ranges of different nano particles have different properties so that we can choose them according to our interest. Pegylated filgrastim have better effects as compared to filgrastim in neutropenia and cause increase in retention time of drug. Moreover pharmacokinetics and pharmacodynamics of Neulasta leave a better impact for treatment over filgrastim in chemotherapy.

REFRENCES

1. Roy, M., et al., *Cellular signalling inhibitors, their formulations and methods thereof*. 2019, Google Patents.
2. Graham, K., et al., *3-amino-1, 5, 6, 7-tetrahydro-4H-indol-4-ones*. 2019, Google Patents.
3. Ferrari, M., *Cancer nanotechnology: opportunities and challenges.* Nature reviews cancer, 2005. **5**(3): p. 161.
4. Finkelman, B.S., et al., *Global patterns in seasonal activity of influenza A/H3N2, A/H1N1, and B from 1997 to 2005: viral coexistence and latitudinal gradients.* PloS one, 2007. **2**(12): p. e1296.
5. Goracci, C., et al., *Evaluation of the adhesion of fiber posts to intraradicular dentin.* OPERATIVE DENTISTRY-UNIVERSITY OF WASHINGTON-, 2005. **30**(5): p. 627.
6. Pilatova, K., et al., *Myeloid-derived suppressor cells (MDSCs) in patients with solid tumors: considerations for granulocyte colony-stimulating factor treatment.* Cancer Immunology, Immunotherapy, 2018. **67**(12): p. 1919-1929.
7. Rouse, G.W., *A cladistic analysis of Siboglinidae Caullery, 1914 (Polychaeta, Annelida): formerly the phyla Pogonophora and Vestimentifera.* Zoological Journal of the Linnean Society, 2001. **132**(1): p. 55-80.
8. Rothman, J.H. and G.K. Schwartz, *Methods and compositions for treating cancer using peptide nucleic acid-based agents*. 2018, Google Patents.
9. Zhang, Z., et al., *A 2010 update of National Land Use/Cover Database of China at 1: 100000 scale using medium spatial resolution satellite images.* Remote sensing of environment, 2014. **149**: p. 142-154.
10. Åkerman, M.E., et al., *Nanocrystal targeting in vivo.* Proceedings of the National Academy of Sciences, 2002. **99**(20): p. 12617-12621.
11. Kim, S.J., et al., *Novel miniature SPR immunosensor equipped with all-in-one multi-microchannel sensor chip for detecting low-molecular-weight analytes.* Biosensors and Bioelectronics, 2007. **23**(5): p. 701-707.

12. Guil, S. and M. Esteller, *DNA methylomes, histone codes and miRNAs: tying it all together.* The international journal of biochemistry & cell biology, 2009. **41**(1): p. 87-95.
13. Parrish, Z.D., M.K. Banks, and A.P. Schwab, *Effectiveness of phytoremediation as a secondary treatment for polycyclic aromatic hydrocarbons (PAHs) in composted soil.* International Journal of Phytoremediation, 2004. **6**(2): p. 119-137.
14. Shrestha, S., et al., *Chemical constituents of Yarsagumba (Ophiocordyceps sinensis (Berk.) Sung et al.), a valued traditional Himalayan medicine.* Nepal Journal of Science and Technology, 2012. **13**(1): p. 43-58.
15. Herrero, A., et al., *Breaking strength of dry fermented sausages and their correlation with texture profile analysis (TPA) and physico-chemical characteristics.* Meat science, 2007. **77**(3): p. 331-338.
16. Jean, C., et al., *Transcriptome analysis of chicken ES, blastodermal and germ cells reveals that chick ES cells are equivalent to mouse ES cells rather than EpiSC.* Stem cell research, 2015. **14**(1): p. 54-67.
17. Abell, G.C., et al., *Effects of estuarine sediment hypoxia on nitrogen fluxes and ammonia oxidizer gene transcription.* FEMS microbiology ecology, 2011. **75**(1): p. 111-122.
18. Omar, F. and T. Alhmiedat, *SUBJECT CLASSIFICATION.*
19. Biswal, B.M. and Z. Yusoff, *Application of Nanotechnology in Cancer Treatment*, in *Engineering Applications of Nanotechnology*. 2017, Springer. p. 269-311.
20. Reyes, Y.D., *Nano-sized delivery systems for potential cancer therapies: Modification of protein formulations to improve pharmacological efficacy*. 2015, University of Puerto Rico, Rio Piedras (Puerto Rico).
21. Basile, L.A., *Uses of il-12 in hematopoietic immunotherapy (hit)*. 2016, Google Patents.
22. Ratner, M.A., D. Ratner, and R. Waser, *Nanotechnology: A gentle introduction to the next big idea*. 2003: Prentice Hall Professional.
23. Kayser, O., A. Lemke, and N. Hernandez-Trejo, *The impact of nanobiotechnology on the development of new drug delivery systems.* Current pharmaceutical biotechnology, 2005. **6**(1): p. 3-5.
24. Lemke, A., A. Kiderlen, and O. Kayser, *Amphotericin b.* Applied microbiology and biotechnology, 2005. **68**(2): p. 151-162.
25. Stokbro, K., J. Taylor, and M. Brandbyge, *Do aviram– ratner diodes rectify?* Journal of the American Chemical Society, 2003. **125**(13): p. 3674-3675.
26. Weiser, J.N., et al., *Antibody-enhanced pneumococcal adherence requires IgA1 protease.* Proceedings of the National Academy of Sciences, 2003. **100**(7): p. 4215-4220.
27. Heath, J.R. and M.A. Ratner, *Molecular electronics.* 2003.
28. Willemze, R., et al., *WHO-EORTC classification for cutaneous lymphomas.* Blood, 2005. **105**(10): p. 3768-3785.
29. Krotkov, N.A., et al., *Aura OMI observations of regional SO 2 and NO 2 pollution changes from 2005 to 2015.* Atmospheric Chemistry and Physics, 2016. **16**(7): p. 4605-4629.
30. Kobayashi, H., et al., *Positive effects of polyethylene glycol conjugation to generation‐4 polyamidoamine dendrimers as macromolecular MR contrast agents.* Magnetic Resonance in Medicine: An Official Journal of

the International Society for Magnetic Resonance in Medicine, 2001. **46**(4): p. 781-788.
31. Kojima, C., et al., *Influence of dendrimer generation and polyethylene glycol length on the biodistribution of PEGylated dendrimers.* International journal of pharmaceutics, 2010. **383**(1-2): p. 293-296.
32. Huang, R.-Q., et al., *Efficient gene delivery targeted to the brain using a transferrin-conjugated polyethyleneglycol-modified polyamidoamine dendrimer.* The FASEB journal, 2007. **21**(4): p. 1117-1125.
33. Esteve-Turrillas, F. and M. de la Guardia, *Smart Materials.* Handbook of Smart Materials in Analytical Chemistry, 2019: p. 1.
34. Ulbricht, M., *Smart Polymeric Membranes with Magnetic Nanoparticles for Switchable Separation*, in *Smart Membranes*. 2019. p. 297-328.
35. Bharath, G., et al., *Shape-controlled rapid synthesis of magnetic nanoparticles and their morphological dependent magnetic and thermal studies for cancer therapy applications.* Materials Research Express, 2019. **6**(6): p. 066104.
36. Oleshkevich, E., et al., *Combining magnetic nanoparticles and icosahedral boron clusters in biocompatible inorganic nanohybrids for cancer therapy.* Nanomedicine: Nanotechnology, Biology and Medicine, 2019.
37. Mauad, T., et al., *Lung pathology in fatal novel human influenza A (H1N1) infection.* American journal of respiratory and critical care medicine, 2010. **181**(1): p. 72-79.
38. da Silva Malheiros, P., D.J. Daroit, and A. Brandelli, *Food applications of liposome-encapsulated antimicrobial peptides.* Trends in Food Science & Technology, 2010. **21**(6): p. 284-292.
39. Immordino, M.L., F. Dosio, and L. Cattel, *Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential.* International journal of nanomedicine, 2006. **1**(3): p. 297.
40. Islas, C., et al., *Preparación y caracterización antimicrobiana de péptidos encapsulados en suportes biopolimericos con una potencial aplicación biotecnológica.* Boletín de Ciencias Agropecuarias del ICAP, 2019. **5**(9): p. 14-16.
41. Jesorka, A. and O. Orwar, *Liposomes: technologies and analytical applications.* Annu. Rev. Anal. Chem., 2008. **1**: p. 801-832.
42. Rasti, B., et al., *Comparative study of the oxidative and physical stability of liposomal and nanoliposomal polyunsaturated fatty acids prepared with conventional and Mozafari methods.* Food chemistry, 2012. **135**(4): p. 2761-2770.
43. Reza Mozafari, M., et al., *Nanoliposomes and their applications in food nanotechnology.* Journal of liposome research, 2008. **18**(4): p. 309-327.
44. Yu, B., R.J. Lee, and L.J. Lee, *Microfluidic methods for production of liposomes.* Methods in enzymology, 2009. **465**: p. 129-141.
45. Patel, G.B., et al., *In vitro assessment of archaeosome stability for developing oral delivery systems.* International journal of pharmaceutics, 2000. **194**(1): p. 39-49.
46. Hsieh, Y.F. and M.J. Silva, *In vivo fatigue loading of the rat ulna induces both bone formation and resorption and leads to time‐related changes in bone mechanical properties and density.* Journal of Orthopaedic Research, 2002. **20**(4): p. 764-771.

47. Hsieh, Y.-F., et al., *The effects of removal and reconstruction of the anterior cruciate ligament on the contact characteristics of the patellofemoral joint.* The American journal of sports medicine, 2002. **30**(1): p. 121-127.
48. Hsieh, Y.F., et al., *Properties of liposomes prepared with various lipids.* Journal of food science, 2002. **67**(8): p. 2808-2813.
49. Wiersig, J. and M. Hentschel, *Combining directional light output and ultralow loss in deformed microdisks.* Physical review letters, 2008. **100**(3): p. 033901.
50. Laridi, R., et al., *Liposome encapsulated nisin Z: optimization, stability and release during milk fermentation.* International dairy journal, 2003. **13**(4): p. 325-336.
51. Were, L.M., et al., *Size, stability, and entrapment efficiency of phospholipid nanocapsules containing polypeptide antimicrobials.* Journal of agricultural and food chemistry, 2003. **51**(27): p. 8073-8079.
52. Ranganathan, A., et al., *Poly (d, l-lactide-co-glycolide)-phospholipid nanocarrier for efficient delivery of macular pigment lutein: absorption pharmacokinetics in mice and antiproliferative effect in Hep G2 cells.* Drug delivery and translational research, 2019. **9**(1): p. 178-191.
53. Abdelmoneem, M.A., et al., *Dual-Targeted Lactoferrin Shell-Oily Core Nanocapsules for Synergistic Targeted/Herbal Therapy of Hepatocellular Carcinoma.* ACS applied materials & interfaces, 2019. **11**(30): p. 26731-26744.
54. O'connor, P., *Shaped attrition resistant particles for co2 capturing and conversion*. 2019, Google Patents.
55. Campuzano, F., R.C. Brown, and J.D. Martínez, *Auger reactors for pyrolysis of biomass and wastes.* Renewable and Sustainable Energy Reviews, 2019. **102**: p. 372-409.
56. Mahato, K., et al., *Gold nanoparticle surface engineering strategies and their applications in biomedicine and diagnostics.* 3 Biotech, 2019. **9**(2): p. 57.
57. Gopalakrishnan, J., *Chimie douce approaches to the synthesis of metastable oxide materials.* Chemistry of Materials, 1995. **7**(7): p. 1265-1275.
58. Kulkarni, S.A., et al., *Perovskite Nanoparticles: Synthesis, Properties, and Novel Applications in Photovoltaics and LEDs.* Small Methods, 2019. **3**(1): p. 1800231.
59. Gallo, A., et al., *Synthesis of eco-compatible bimetallic silver/iron nanoparticles for water remediation and reactivity assessment on bromophenol blue.* Journal of cleaner production, 2019. **211**: p. 1367-1374.
60. Atchudan, R., et al., *Synthesis of high-quality carbon nanotubes by using monodisperse spherical mesoporous silica encapsulating iron oxide nanoparticles.* Korean Journal of Chemical Engineering, 2019. **36**(1): p. 157-165.
61. Hatami, R., et al., *Preparation of amino silane magnetic nanocomposite by the sol–gel process and investigation of its antibacterial activity.* Micro & Nano Letters, 2019. **14**(2): p. 196-201.
62. Kumar, K.A., et al., *Sol-gel synthesized plasmonic nanoparticles and their integration into dye sensitized solar cells.* Applied Surface Science, 2019.
63. Innocenzi, P., *The Precursors of the Sol-Gel Process*, in *The Sol-to-Gel Transition*. 2019, Springer. p. 7-19.

64. Hochman, J.S., et al., *Current spectrum of cardiogenic shock and effect of early revascularization on mortality: results of an international registry.* Circulation, 1995. **91**(3): p. 873-881.
65. Hench, L.L. and J.K. West, *The sol-gel process.* Chemical reviews, 1990. **90**(1): p. 33-72.
66. Klein, L.E., *Shaftesbury and the culture of politeness: moral discourse and cultural politics in early eighteenth-century England*. 1994: Cambridge University Press.
67. Richards, C., et al., *Assessment of Antifouling Potential of Novel Transparent Sol Gel Coatings for Application in the Marine Environment.* Molecules, 2019. **24**(16): p. 2983.
68. Angelova, T., et al., *Study of potential biomedical application of sol-gel derived Zn-doped SiO2-hydroxypropyl cellulose nanohybrids.* Materials Science and Engineering: C, 2019. **100**: p. 608-615.
69. Corriu, R. and T.A. Nguyên, *Molecular chemistry of sol-gel derived nanomaterials*. 2009: Wiley Online Library.
70. Hwang, T.-Y., et al., *Synthesis and magnetic properties of Sm2Co17 particles using salt-assisted spray pyrolysis and a reduction-diffusion process.* Applied Surface Science, 2019. **475**: p. 986-989.
71. Hwangbo, Y. and Y.-I. Lee, *Facile synthesis of zirconia nanoparticles using a salt-assisted ultrasonic spray pyrolysis combined with a citrate precursor method.* Journal of Alloys and Compounds, 2019. **771**: p. 821-826.
72. Xia, B., I.W. Lenggoro, and K. Okuyama, *Novel route to nanoparticle synthesis by salt‐assisted aerosol decomposition.* Advanced Materials, 2001. **13**(20): p. 1579-1582.
73. Ji, M.-J., et al., *Synthesis and Optical Property of TiO 2 Nanoparticles Using a Salt-assisted Ultrasonic Spray Pyrolysis Process.* Journal of Korean Powder Metallurgy Institute, 2019. **26**(1): p. 34-39.
74. Zhao, J., C. Lei, and Y. Du, *Influence of precursor and salt assisted calcination on magnetic properties of Sr-ferrites.* Journal of Materials Science: Materials in Electronics, 2019: p. 1-6.
75. Zhang, J. and C.M. Megaridis, *Soot suppression by ferrocene in laminar ethylene/air nonpremixed flames.* Combustion and Flame, 1996. **105**(4): p. 528-540.
76. Christy, S.R., et al., *Simple Combustion Synthesis, Structural, Morphological, Optical and Catalytic Properties of ZnO Nanoparticles.* Journal of nanoscience and nanotechnology, 2019. **19**(6): p. 3564-3570.
77. Kombaiah, K., et al., *Catalytic studies of NiFe2O4 nanoparticles prepared by conventional and microwave combustion method.* Materials Chemistry and Physics, 2019. **221**: p. 11-28.
78. Hong, R., et al., *Magnetic field synthesis of Fe3O4 nanoparticles used as a precursor of ferrofluids.* Journal of magnetism and magnetic materials, 2007. **310**(1): p. 37-47.
79. Arenas-Alatorre, J., et al., *Synthesis and characterization of iron oxide nanoparticles grown via a non-conventional chemical method using an external magnetic field.* Materials Letters, 2019. **242**: p. 13-16.
80. Devi, H.S., et al., *Green synthesis of iron oxide nanoparticles using Platanus orientalis leaf extract for antifungal activity.* Green Processing and Synthesis, 2019. **8**(1): p. 38-45.

81. Demirezen, D.A., et al., *Green synthesis and characterization of iron oxide nanoparticles using Ficus carica (common fig) dried fruit extract.* Journal of bioscience and bioengineering, 2019. **127**(2): p. 241-245.
82. Henam, S.D., et al., *Microwave synthesis of nanoparticles and their antifungal activities.* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2019. **213**: p. 337-341.
83. Kerroum, M.A., et al., *The effect of basic pH on the elaboration of ZnFe2O4 nanoparticles by co-precipitation method: Structural, magnetic and hyperthermia characterization.* Journal of Magnetism and Magnetic Materials, 2019. **478**: p. 239-246.
84. Tartaj, P., et al., *The preparation of magnetic nanoparticles for applications in biomedicine.* Journal of physics D: Applied physics, 2003. **36**(13): p. R182.
85. Pedro, T.M., J.M. MacKeown, and S.A. Norris, *Variety and total number of food items recorded by a true longitudinal group of urban black South African children at five interceptions between 1995 and 2003: the Birth-to-Twenty (Bt20) Study.* Public health nutrition, 2008. **11**(6): p. 616-623.
86. Asgari, S., et al., *Microemulsions as nanoreactors for synthesis of biopolymer nanoparticles.* Trends in Food Science & Technology, 2019.
87. Tarek, M. and A. El-Aziz. *Synthesis and Characterization of Silver Nanoparticles Using Simple Polyol Method*. in *TMS 2019 148th Annual Meeting & Exhibition Supplemental Proceedings*. 2019. Springer.
88. Wu, Z., et al., *Palladium nanostructures with well-controlled morphologies obtained by one-pot and one-step polyol method.* Journal of Crystal Growth, 2019. **521**: p. 34-40.
89. McBride, A., et al., *Abstract P4-12-07: Cost-minimization of chemotherapy-induced (febrile) neutropenia prophylaxis with biosimilar ZARXIO® over NEUPOGEN®, NEULASTA®, and NEULASTA/ONPRO®: Breast cancer case study*. 2018, AACR.
90. Schmidt, S.H., et al., *Pegfilgrastim (Neulasta®) Vs. Tbo-Filgrastim (Granix®) Post Autologous Transplant.* Biology of Blood and Marrow Transplantation, 2018. **24**(3): p. S331-S332.
91. Legesse, B., et al., *Neulasta Regimen for the Hematopoietic Acute Radiation Syndrome: Effects Beyond Neutrophil Recovery.* International Journal of Radiation Oncology* Biology* Physics, 2019. **103**(4): p. 935-944.
92. Heil, G., et al., *A randomized, double-blind, placebo-controlled, phase III study of filgrastim in remission induction and consolidation therapy for adults with de novo acute myeloid leukemia.* Blood, 1997. **90**(12): p. 4710-4718.
93. Kluijtmans, L.A., et al., *Thermolabile methylenetetrahydrofolate reductase in coronary artery disease.* Circulation, 1997. **96**(8): p. 2573-2577.
94. Roskos, L.K., et al., *Pharmacology of filgrastim (r-metHuG-CSF).*) Filgrastim (r-methug-csf) in clinical practice 2nd ed. edn. Marcel Dekker, New York, 1998: p. 51-711998.
95. Waller, C., et al., *A pharmacokinetics and pharmacodynamics equivalence trial of proposed pegfilgrastim biosimilar, MYL-1401H vs EU neulasta® and US neulasta®.* Annals of Oncology, 2016. **27**(suppl_6).
96. Lickliter, J.D., et al., *Pharmacokinetic/pharmacodynamic assessment of a proposed biosimilar MSB11455 versus the currently licensed pegfilgrastim:*

A randomized, double-blind trial. 2019, American Society of Clinical Oncology.
97. Roth, K., et al., *Demonstration of pharmacokinetic and pharmacodynamic equivalence in healthy volunteers for B12019, a new proposed pegfilgrastim biosimilar*. 2016, Am Soc Hematology.
98. Lipiäinen, T., et al., *Formulation and stability of cytokine therapeutics.* Journal of pharmaceutical sciences, 2015. **104**(2): p. 307-326.
99. Alshora, D.H., M.A. Ibrahim, and F.K. Alanazi, *Nanotechnology from particle size reduction to enhancing aqueous solubility*, in *Surface Chemistry of Nanobiomaterials*. 2016, Elsevier. p. 163-191.
100. Swierczewska, M., K.C. Lee, and S. Lee, *What is the future of PEGylated therapies?* 2015, Taylor & Francis.
101. Strohl, W.R., *Fusion proteins for half-life extension of biologics as a strategy to make biobetters.* BioDrugs, 2015. **29**(4): p. 215-239.
102. Larsen, M.T., et al., *Cellular recycling-driven in vivo half-life extension using recombinant albumin fusions tuned for neonatal Fc receptor (FcRn) engagement.* Journal of controlled release, 2018. **287**: p. 132-141.
103. van Witteloostuijn, S.B., S.L. Pedersen, and K.J. Jensen, *Half‐life extension of biopharmaceuticals using chemical methods: alternatives to PEGylation.* ChemMedChem, 2016. **11**(22): p. 2474-2495.
104. Zaman, R., et al., *Current strategies in extending half-lives of therapeutic proteins.* Journal of controlled release, 2019.
105. Relias, V., N. Lyons, and K.A. Sprague, *Pegfilgrastim in Autologous Stem Cell Transplant.* Biology of Blood and Marrow Transplantation, 2016. **22**(3): p. S456-S457.
106. Siefker-Radtke, A.O., et al., *Front-line treatment with gemcitabine, paclitaxel, and doxorubicin for patients with unresectable or metastatic urothelial cancer and poor renal function: final results from a phase II study.* Urology, 2016. **89**: p. 83-89.
107. Sharma, R. and V. Sharma, *IDDF2019-ABS-0208 Exciting results with injection darbepoietin alfa and pegfilgrastim in patients with decompensated liver cirrhosis*. 2019, BMJ Publishing Group.
108. Paglialunga, S., et al., *Update and trends on pharmacokinetic studies in patients with impaired renal function: practical insight into application of the FDA and EMA guidelines.* Expert review of clinical pharmacology, 2017. **10**(3): p. 273-283.
109. Cheema, A., et al., *A Metabolomic and lipidomic serum signature from nonhuman primates administered with a promising radiation countermeasure, gamma-tocotrienol.* International journal of molecular sciences, 2017. **19**(1): p. 79.
110. Guinigundo, A.S., et al., *A Randomized, Single-Blind Study Evaluating the Effect of a Bone Pain Education Video on Reported Bone Pain in Patients with Breast Cancer Receiving Chemotherapy and Pegfilgrastim.* Pain Management Nursing, 2018. **19**(6): p. 693-706.
111. Butcher, N.J., G.M. Mortimer, and R.F. Minchin, *Drug delivery: Unravelling the stealth effect.* Nature nanotechnology, 2016. **11**(4): p. 310.
112. Brand, W., et al., *Nanomedicinal products: a survey on specific toxicity and side effects.* International journal of nanomedicine, 2017. **12**: p. 6107.

113. Carlson, L.E., et al., *The ONE‐MIND Study: Rationale and protocol for assessing the effects of ONlinE MINDfulness‐based cancer recovery for the prevention of fatigue and other common side effects during chemotherapy.* European journal of cancer care, 2019: p. e13074.
114. Oakley, P.A., *On the Side Effects of 'Pain'and 'Dehydration'in the Top 20 Selling Pharmaceuticals of 2017.* Journal of Pharmaceutical Research International, 2018: p. 1-9.
115. Price, P.W. and A.L. DiCarlo, *Challenges and benefits of repurposing licensed/approved/cleared products for a radiation indication.* Radiation research, 2018. **190**(6): p. 654-658.
116. DiCarlo, A.L., et al., *Challenges and benefits of repurposing products for use during a radiation public health emergency: Lessons learned from biological threats and other disease treatments.* Radiation research, 2018. **190**(6): p. 659-676.
117. Farese, A.M., et al., *Efficacy of Neulasta or Neupogen on H-ARS and GI-ARS Mortality and Hematopoietic Recovery in Nonhuman Primates After 10-Gy Irradiation With 2.5% Bone Marrow Sparing.* Health physics, 2019. **116**(3): p. 339-353.
118. Kirshner, J.J., et al., *NOLAN: a randomized, phase 2 study to estimate the effect of prophylactic naproxen or loratadine vs no prophylactic treatment on bone pain in patients with early-stage breast cancer receiving chemotherapy and pegfilgrastim.* Supportive Care in Cancer, 2018. **26**(4): p. 1323-1334.
119. Rando, A., et al., *Granulocyte colony-stimulating factor ameliorates skeletal muscle dysfunction in amyotrophic lateral sclerosis mice and improves proliferation of SOD1-G93A myoblasts in vitro.* Neurodegenerative Diseases, 2017. **17**(1): p. 1-13.
120. Demontis, G.C., et al., *Human pathophysiological adaptations to the space environment.* Frontiers in physiology, 2017. **8**: p. 547.
121. Malhotra, B., et al., *14 Polymers Targeting Habitual Diseases.* Natural Polymers for Drug Delivery, 2016: p. 171.
122. Weber, G.F., *Protection from Adverse Effects*, in *Molecular Therapies of Cancer*. 2015, Springer. p. 425-443.
123. Thwala, L.N., V. Préat, and N.S. Csaba, *Emerging delivery platforms for mucosal administration of biopharmaceuticals: a critical update on nasal, pulmonary and oral routes.* Expert opinion on drug delivery, 2017. **14**(1): p. 23-36.
124. Singh, J., et al., *Polymer drug conjugates: recent advancements in various diseases.* Current pharmaceutical design, 2016. **22**(19): p. 2821-2843.
125. Soriano, J.L., et al., *Therapy for prevention and treatment of skin ionizing radiation damage: a review.* International journal of radiation biology, 2019. **95**(5): p. 537-553.
126. Alperstein, N.M., *Skin Wars: Building Mediated Social Connections to Promote Prescription Drugs*, in *Celebrity and Mediated Social Connections*. 2019, Springer. p. 161-193.
127. Carnell, L.S., et al., *Medical Countermeasures for Radiation Induced Health Effects: Report of an Interagency Panel Session Held at the NASA Human*

Research Program Investigator's Workshop, January 26, 2017. International Journal of Radiation Biology, 2019(just-accepted): p. 1-23.
128. Schaue, D. and W.H. McBride, *Are animal models a necessity for acute radiation syndrome drug discovery?* 2019, Taylor & Francis.
129. Barthel, J. and N. Sarigul-Klijn, *A review of radiation shielding needs and concepts for space voyages beyond Earth's magnetic influence.* Progress in Aerospace Sciences, 2019: p. 100553.
130. Arora, G., *Nanocarrier: A Boom or a Bane in the Medical Industry.* Acta Scientific Pharmaceutical Sciences, 2019. **3**: p. 08-23.
131. Patil, S., et al., *Formulation of Therapeutic Proteins: Strategies for Developing Oral Protein Formulations.* Innovative Drug Formulations: Design and Development at Early Stage, 2019.
132. Sharan, K., *Pain in Hematologic Malignancies*, in *Essentials of Interventional Cancer Pain Management*. 2019, Springer. p. 123-131.
133. Takuwa, H., et al., *Pegfilgrastim-induced fatigue and leukocytosis improved following dose reduction in a young patient with breast cancer: A case report.* Molecular and Clinical Oncology, 2019. **11**(4): p. 371-375.
134. Kosaka, T., et al., *Impact of pegfilgrastim as primary prophylaxis for metastatic castration-resistant prostate cancer patients undergoing cabazitaxel treatment: an open-label study in Japan.* Japanese Journal of Clinical Oncology, 2019.
135. Gu, L., et al., *The effects of granulocyte colony-stimulating factor on MR images of bone marrow.* Skeletal radiology, 2019. **48**(2): p. 209-218.
136. Wessels, H., et al., *Pharmacodynamics, safety, and immunogenicity of Pelmeg®, a pegfilgrastim biosimilar in healthy subjects.* Pharmacology research & perspectives, 2019. **7**(5): p. e00507.
137. Shepard, H.M., et al., *Adverse side-effects associated with administration of an anti-hyaluronan agent and methods for ameliorating or preventing the side-effects*. 2019, Google Patents.
138. Burke, M.S., et al., *Presurgical induction chemotherapy for squamous cell carcinoma of the tonsil.* The Laryngoscope, 2019.
139. González-Cavero, L., et al., *Desensitization to Filgrastim in a 2-Year-Old Girl With a Vaginal Endodermal Sinus Tumor.* Journal of investigational allergology & clinical immunology, 2019. **29**(4): p. 329-330.
140. Carvalho, A., A.R. Fernandes, and P.V. Baptista, *Nanoparticles as Delivery Systems in Cancer Therapy: Focus on Gold Nanoparticles and Drugs*, in *Applications of Targeted Nano Drugs and Delivery Systems*. 2019, Elsevier. p. 257-295.
141. Çalış, S., et al., *Nanopharmaceuticals as Drug-Delivery Systems: For, Against, and Current Applications*, in *Nanocarriers for Drug Delivery*. 2019, Elsevier. p. 133-154.
142. Rana, V. and R. Sharma, *Recent Advances in Development of Nano Drug Delivery*, in *Applications of Targeted Nano Drugs and Delivery Systems*. 2019, Elsevier. p. 93-131.
143. Elzahhar, P., et al., *Bioconjugation in Drug Delivery: Practical Perspectives and Future Perceptions*, in *Pharmaceutical Nanotechnology*. 2019, Springer. p. 125-182.

144. Yang, B.-B., et al., *Polyethylene glycol modification of filgrastim results in decreased renal clearance of the protein in rats.* Journal of pharmaceutical sciences, 2004. **93**(5): p. 1367-1373.
145. Zheng, P.-S., et al., *PG-M/versican binds to P-selectin glycoprotein ligand-1 and mediates leukocyte aggregation.* Journal of cell science, 2004. **117**(24): p. 5887-5895.
146. Bi, R., et al., *Alternating current electrical field effects on lettuce (Lactuca sativa) growing in hydroponic culture with and without cadmium contamination.* Journal of applied electrochemistry, 2010. **40**(6): p. 1217-1223.
147. Bi, E., *Lord of the ring.* Nature Reviews Molecular Cell Biology, 2010. **11**(9): p. 606.
148. Roskos, L.K., et al., *Pharmacokinetic/pharmacodynamic modeling of pegfilgrastim in healthy subjects.* The Journal of Clinical Pharmacology, 2006. **46**(7): p. 747-757.
149. Yang, B.-B., et al., *Pharmacokinetic and pharmacodynamic perspectives on the clinical drug development of panitumumab.* Clinical pharmacokinetics, 2010. **49**(11): p. 729-740.
150. Johnston, E., et al., *Randomized, dose-escalation study of SD/01 compared with daily filgrastim in patients receiving chemotherapy.* Journal of Clinical Oncology, 2000. **18**(13): p. 2522-2528.
151. Holmes, F., 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.* Journal of Clinical Oncology, 2002. **20**(3): p. 727-731.
152. Holmes, F., 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.* Annals of Oncology, 2002. **13**(6): p. 903-909.
153. Green, M., et al., *A randomized double-blind multicenter phase III study of fixed-dose single-administration pegfilgrastim versus daily filgrastim in patients receiving myelosuppressive chemotherapy.* Annals of Oncology, 2003. **14**(1): p. 29-35.
154. Pramuanyat, N., K.N. Nakorn, and K. Rojviboonchai. *Preliminary study of reliable broadcast protocol on 802.11 p public transport testbed*. in *2015 12th International Conference on Electrical Engineering/Electronics, Computer, Telecommunications and Information Technology (ECTI-CON)*. 2015. IEEE.
155. Wendelin, G., et al., *Once-per-cycle pegfilgrastim versus daily filgrastim in pediatric patients with Ewing sarcoma.* Journal of pediatric hematology/oncology, 2005. **27**(8): p. 449-451.
156. MacDonald, M.E., et al., *A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes.* Cell, 1993. **72**(6): p. 971-983.
157. Albone, E. and M. Fox, *Anal gland secretion of the red fox.* Nature, 1971. **233**(5321): p. 569.
158. Vose, J.M., et al., *Randomized, multicenter, open-label study of pegfilgrastim compared with daily filgrastim after chemotherapy for lymphoma.* Journal of Clinical Oncology, 2003. **21**(3): p. 514-519.

159. Slany, M. and I. Pavlik, *Molecular detection of nontuberculous mycobacteria: advantages and limits of a broad-range sequencing approach.* Journal of molecular microbiology and biotechnology, 2012. **22**(4): p. 268-276.
160. Molineux, G. and T. Dexter, *Biology of G-CSF.* Filgrastim (r-metHuG-CSF) in Clinical Practice. New York: Marcel Dekker, Inc, 1994: p. 1-21.
161. Ryan, G.R., et al., *Rescue of the colony-stimulating factor 1 (CSF-1)–nullizygous mouse (Csf1 op/Csf1 op) phenotype with a CSF-1 transgene and identification of sites of local CSF-1 synthesis.* Blood, 2001. **98**(1): p. 74-84.
162. Demetri, G.D. and J.D. Griffin, *Granulocyte colony-stimulating factor and its receptor.* Blood, 1991. **78**(11): p. 2791-2808.
163. Avalos, B.R., *Molecular analysis of the granulocyte colony-stimulating factor receptor.* Blood, 1996. **88**(3): p. 761-777.
164. Klomp, L.W., et al., *Molecular characterization of 3-phosphoglycerate dehydrogenase deficiency—a neurometabolic disorder associated with reduced L-serine biosynthesis.* The American Journal of Human Genetics, 2000. **67**(6): p. 1389-1399.
165. Schloss, P. and D.C. Williams, *The serotonin transporter: a primary target for antidepressant drugs.* Journal of psychopharmacology, 1998. **12**(2): p. 115-121.
166. Dong, F., et al., *Identification of a nonsense mutation in the granulocyte-colony-stimulating factor receptor in severe congenital neutropenia.* Proceedings of the National Academy of Sciences, 1994. **91**(10): p. 4480-4484.
167. Dong, F., E.P. Gogol, and P.H. von Hippel, *The phage T4-coded DNA replication helicase (gp41) forms a hexamer upon activation by nucleoside triphosphate.* Journal of Biological Chemistry, 1995. **270**(13): p. 7462-7473.
168. Bass, B.M., et al., *Predicting unit performance by assessing transformational and transactional leadership.* Journal of applied psychology, 2003. **88**(2): p. 207.
169. Starikova, Z., et al., *Synthesis and crystal structure of the double barium-titanium methoxide [Ba2Ti4O (OMe) 18 (MeOH) 7]· MeOH.* Polyhedron, 1997. **16**(24): p. 4347-4351.
170. Dong, F., et al., *Mutations in the granulocyte colony-stimulating factor receptor gene in patients with severe congenital neutropenia.* Leukemia, 1997. **11**(1): p. 120.
171. Dong, J. and D.B. Wagner, *Paternally inherited chloroplast polymorphism in Pinus: estimation of diversity and population subdivision, and tests of disequilibrium with a maternally inherited mitochondrial polymorphism.* Genetics, 1994. **136**(3): p. 1187-1194.
172. Hermans, E., S. Nahorski, and R. Challiss, *Reversible and non-competitive antagonist profile of CPCCOEt at the human type 1α metabotropic glutamate receptor.* Neuropharmacology, 1998. **37**(12): p. 1645-1647.
173. Bonenkamp, J., et al., *Extended lymph-node dissection for gastric cancer.* New England Journal of Medicine, 1999. **340**(12): p. 908-914.
174. Dong, X.M., J.-F. Revol, and D.G. Gray, *Effect of microcrystallite preparation conditions on the formation of colloid crystals of cellulose.* Cellulose, 1998. **5**(1): p. 19-32.

175. Innis-Shelton, R. and L.J. Costa, *Sources of Cells for Hematopoietic Cell Transplantation: Practical Aspects of Hematopoietic Cell Collection*, in *Hematopoietic Cell Transplantation for Malignant Conditions*. 2019, Elsevier. p. 73-84.
176. Oliveira, M., et al., *Comprehensive analysis of the influence of G-CSF on the biodistribution of 18 F-FDG in lymphoma patients: insights for PET/CT scheduling.* EJNMMI research, 2019. **9**(1): p. 1-10.
177. Wong, K., et al., *Pharmacodynamics of Romiplostim Alone and in Combination with Pegfilgrastim on Acute Radiation-Induced Thrombocytopenia and Neutropenia in Non-Human Primates.* International Journal of Radiation Biology, 2019(just-accepted): p. 1-30.
178. Dong, F., et al., *Investigation of the effective oxygen storage and release performances on the Pt/CeO2-ZrO2 catalysts by breakthrough method.* Catalysis Today, 2019. **332**: p. 259-266.
179. Deshpande, R., et al., *Estradiol down‐regulates LPS‐induced cytokine production and NFkB activation in murine macrophages.* American journal of reproductive immunology, 1997. **38**(1): p. 46-54.
180. Mora-Garcia, P. and K.M. Sakamoto, *Granulocyte Colony-stimulating Factor Induces egr-1Up-regulation through Interaction of Serum Response Element-binding Proteins.* Journal of Biological Chemistry, 2000. **275**(29): p. 22418-22426.
181. Duerr, E.-M., et al., *Defining molecular classifications and targets in gastroenteropancreatic neuroendocrine tumors through DNA microarray analysis.* Endocrine-related cancer, 2008. **15**(1): p. 243.
182. O'Reilly, M.S., et al., *Endostatin: an endogenous inhibitor of angiogenesis and tumor growth.* cell, 1997. **88**(2): p. 277-285.
183. Nicholson, R., et al., *Pure anti-oestrogens (ICI 164384 and ICI 182780) and breast cancer: is the attainment of complete oestrogen withdrawal worthwhile?* Endocrine-Related Cancer, 1994. **1**(4): p. 5-17.
184. De Koning, K. and M. Martin, *Participatory research in health: Issues and experiences*. 1996: Zed Books.
185. Corey, A.T., *Mechanics of immiscible fluids in porous media*. 1994: Water Resources Publication.
186. Corey-Bloom, J., *A randomized trial evaluating the efficacy and safety of ENA 713 (rivastigmine tartrate), a new acetylcholinesterase inhibitor, in patients with mild to moderately severe Alzheimer's disease.* Int J Geriatr Psyopharmacol, 1998. **1**: p. 55-65.
187. Dong, B. and R.H. Silverman, *2-5A-dependent RNase molecules dimerize during activation by 2-5A.* Journal of Biological Chemistry, 1995. **270**(8): p. 4133-4137.