A Randomized clinical trial to compare Tinagrast, a product of AryaTinaGene Company, with Neupogen, a reference medicine produced by Amgen company, to prevent febrile neutropenia following chemotherapy

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Contents:

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Study advisory group and their responsibilities:

Responsibility	Expertise	Full Name of Specialist
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Study Financial Support

Established in 2002, AryaTinaGeneLtd.,a research-production company, bearing the registration number 7550 and national ID 10102706497, came into operation in 2014. This company has provided this study with financial support in order for the drug to be introduced into the market.
Appointed Scientific-Executive Committee

Expertise	Full Name
Genetics and Immunology Specialist	Dr. Majid Shahbazi
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Changes in registration process during the study

While this study started *prior to marketing, the drug name changed from* filgrastim to the generic name of the drug i.e. Filgrastim; this change was done according to Iran FDA approval letter No. 665/139468 on July 10, 2013. On February 17, 2014, Tinagrast was registered as the trade name of the drug and it entered the clinical study after legal processes. In order for the pre-filled syringes of Tinagrast to be used in the clinical study, the batch No. changed from AH001 to AH004 according to Iran FDA approval letter No. 665/139468 on June 22, 2015.

Current drug situation in Iran

According to statistics, the use of Filgrastim showed an increase from 2001 to 2012. Tinagrast, with a thoroughly similar formulation of reference product, entered the market in March 2014 after the conduction of clinical trial and receiving Iran FDA approval. It succeeded in building trust amongst a lot of specialists and patients, *holding 41.8%* of the *market share* according to the statistics released in the first six month of 1394 Hijri year, between March 2015 and September 2015.

Abstract

Since the early 1940s, chemotherapy has been implemented as one of the therapeutic strategies for cancer treatmen. Despite the beneficial effects (destroying cancer cells) of chemotherapy,unfortuantely, other alive and natural texture and organs of body, specifically fast dividing cells , have also been damaged in varying degrees. Theoretically, all chemotherapy regimens can cause immune system and bone marrow suppression. This suppression may lead to low white blood cell count, low red blood cell count ,and low platelet count. G-CSF (a recombinant form), bearing Filgrastim as the generic name, is known and recognised as an effective and significant treatment to prevent a decrerase in blood neutrophils. Therefore, the present clinical trial has been designed aiming at studying the efficacy of Filgrastim (an AryaTinaGene`s product, bearing Tinagrast as the generic name) in the prevention and treatment of neutropenia following chemotherapy in comparison with reference product.

The present study has been designed as a randomized, double-blind, multicenter, parallel trial. Patients diagnosed with breast cancer who fulfilled the inclusion criteria were assigned to two groups at random. After randomization was performed,92 patients fulfilling the inclusion criteria entered the study and were rendomly assigned to one of these two groups: 49 patients in Neupogen group and 43 patients in Tinagrast group. Every patient was given daily 5 mcg of Neupogen per kilogram *24* hours after every cycle of chemotherapy *(*each cycle of chemotherapy takes 21 days or three weeks*)*.

Findings of the present trial showed that, in phase 1,2,and 3 clinical trials, over 95 percent of participants reached normal levels of Neutrophil in two clinical control groups (as an index,

absolute neutrophils count is 1,500 per cubic millimeter of blood). It was only in phase 4 that normal neutrophil count in participants of two groups was lower than that of other previous phases; no statistically significant difference was observed between these indexes (in both groups of Neupogen and Tinagrast ,respectively, 76.7 and 74.4 percent of participants` neutrophil count was normal, i.e. $p>0.05$).

Statement of the problem

According to world cancer report released by American Cancer Society, in 2012, between two genders, 14.1 million people were diagnosed with cancer, among which 8 million cases (82 per cent) are in underdevelopment countries (this excludes non-melanoma skin cancer to which no exact statistics is assigned yet). Cancer is the second most common cause of death after cardiovascular events. Cancers of the respiratory tract (lungs, trachea, bronchi) in men and breast cancer in women is on top of the world. Cancer death cases were reported to be as many as 8.2 million in 2012, showing a high frequency.

Since the early 1940s, chemotherapy has been implemented as one of the therapeutic strategies for cancer treatmen. Since then, there have been so many advancements in this method that today, chemotherapy ,along with radiation therapy and surgery, one of the most common treatments for malignant cancers. Despite the beneficial effects (destroying cancer cells) of chemotherapy,unfortuantely, other alive and natural texture and organs of body, specifically fast dividing cells , have also been damaged in varying degrees. Various chemotherapy protocols to treat different cancers are developed according to the basic principle of maximizing destroying cancer cells and to minimize the adverse effects on normal, healthy cells. Thus, according to this principle, different chemotherapy protocols for the treatment of various cancers were developed, and these protocols are being optimised with the development of more efficient drugs used in chemotherapy.

Adverse effects of chemotherapy drugs are divided into two groups: common side effects and organs-specific effects. Common side effects includ suppression of the immune system, fatigue, thrombocytopenia and a tendency to bleed, gastrointestinal symptoms (nausea and vomiting),

Mucosal inflammation and loss of wax [2]. Theoretically, all regimens of chemotherapy can cause immune system and bone marrow suppression, low white blood cell count, low red blood cell count and low platelet count.

In different organ cancers, the frequency of various chemotherapy administration methods varies, depending on the type and nature of the cancer, progression stage of cancer, and the patients' clinical condition. In a study conducted in order to to assess the treatment pattern of women aged 65 and older diagnosed with breast cancer, it was showen that the frequency of chemotherapy administration increased as the disease progresses – the administration frequency of this methods in stages 1, 2, 3 and 4 were 5.1%, 19.5%, 33.9%, and 35.2%, respectively [4]. In another randomized controlled study, using breast cancer care system database of differect states of the US, about ten thousand women age 20 years and older diagnosed with breast cancer were studied. The findings of this study showed that the administration of chemotherapy alone (without the use of hormone therapy such as tamoxifen) in different groups of patients varied between about 48% to nearly 60%. In addition, the frequency of using different chemotherapy regimens increased between 1987 and 2000 [5].

The risk of occurrence of bone marrow suppression and neutropenia in patients diagnosed with cancer varies. In a study on 35 thousand women aged 65 and older diagnosed with breast cancer using breast cancer care system database in in the US, it was found that more than 9% of women receiving cancer chemotherapy regimens experience hospitalization due to fever, neutropenia, thrombocytopenia (or other systemic effects). While, hospitalization due to these disorders without the use of chemotherapy in women was aboiut 0.5%. In addition, the rate of hospitalization due to these adverse effects in stages 1, 2, 3 ,and 4 was reported to be 6.3%, 8.1%, 12.3% and 13.2, respectively [6].

In another study using breast cancer care system database in the US in 15 different geographic areas, about 10 thousand women with various stages of ovarian cancer in terms of incidence of hospitalization due to bone marrow suppression in the period between 1991 and 2002 were assessed. In this study, about 65.7% of patients who were assessed, received a different chemotherapy regimens. Risk factors for hospitalization due to infection or bone marrow suppression in these patients included chemotherapy regimen containing no- platinum compounds compounds (compared to platinum compounds), comorbidity score, and age [7].

In a comprehensive study carried out on about 65 thousand women with breast cancer and about 7500 women with ovarian cancer aged 65 years or older in 16 different areas in the US (based on the cancer care system), the occurrence of bone marrow suppression associated with chemotherapy (regardless of whether or not resulting in hospitalization) was assessed as the main outcome of the study. According to this study, the incidence of short-term neutropenia (less than 3 months) in different regimens of chemotherapy varies between 11.0 to 47.7 per 1000 person-year for breast cancer and between 25.2 to 80.9 per 1000 person-years for ovarian cancer. The incidence of long-term neutropenia (within 3 months) in different regimens of chemotherapy in breast cancer varies between 18.8 to 40.6 per 1000 person-years for ovarian cancer and between 35.3 to 109.1 per 1000 person-years. In addition, in most chemotherapy regimens, the dose – response relationship between different values or cycles of chemotherapy with bone marrow toxicity was observed [8]. In many cancers, including colorectal cancer, [10] lung cancer, [11] and breast cancer [12], a decrease in the dose of chemotherapy or a delay in the next cycle [9] leads to the deterioration in the prognosis of patients.

Granulocyte colony-stimulating growth factor is a glycoprotein, growth factor, or cytokine secreted in the body by endothelial cells, macrophages ,and other cells of the immune system; it also causes differentiation of granulocytes by stimulation of the bone marrow .This factor is also known as known as Hematopoietic Stem Cell (HSCs) mobilization and causes stem cells to enter peripheral blood.

In addition to the above-mentioned major effects , G-CSF is a neurotropic factor and can produce new nerve cells and prevent them from apoptosis, affecting the central nervous system. Emphasising on this feature, scientists are trying to find new ways for the treatment of nervous system diseases [13-15].
In 1983, mouse G-CSF was developed for the first in Australia, followed by the development of its human counter part in Japan, America, and Germany in 1986.

For more than two decades, this indispensable factor has been used to prevent and treat chemotherapy-induced neutropenia [19]. It can also reduce the risk of neutropenia with or without fever during chemotherapy, decrease infection, reduce the need for antibiotics, and accelerate the

improvement of neutrophils [20-24]. It is worth noting that in patients receiving it, factors of dose reduction or delay in chemotherapy decreases [25, 26].

After this factor was put into mass production, gradually, it entered the pharmaceutical market in different countries and , is being routinely used to treat cancer patients, especially those who are suffering from complications of neutropenia after chemotherapy. The first factor were produced first by Amgen under the brand name of Neupogen. Today, many generic products of this factor are available in the pharmaceutical markets of Europe and Australia. Filgrastim is a human recombinant made by factors in E. coli.

Most researches published worldwide have been perfomed on Filgrastim (under the trade name of Neupogen). Neulasta is also the brand name of PEGylated or PEG-Filgrastim. In 1991, Neupogen produced by Amgen was approved by the Food and Drug Administration of America as prophylaxis of neutropenia in cancer patients after chemotherapy . It should laso be mentioed that, since 1989, Roche in Switzerland had monopolised the sales of these two drugs (under license) in several countries, including the Middle East, which was was handed back to Amgen on January 2014 [27, 28].

Lenograstim is also another form of human recombinant of granulocyte growth stimulating factor made in Chinese hamster ovary cells [29]. American Society of Clinical Oncology (ASCO) first published the guideline to apply these factors in 1994. The application guide is in the form of primary prevention (in the first cycle), when the anticipated risk of fever and neutropenia after chemotherapy gets more than 40%. It was reported that, in 2006, the rate changed to 20% [30, 31]

In 2007, in American Journal of Clinical Oncology, a report on the survey findings of 17 controlled trials on 3493 cancer patients after chemotherapy was announced; it was proved that the fever and neutropenia and death following infection reduced after using GCSF as a preventer [25].

In contrast, in the same year, in another study conducted to evaluate the findings of 148 trials , no effect was placed for growth factor in reducing the mortality rate for patients in similar

circumstances, while, assuming that reduced incidence of infection after taking this factor was true [32].

After surveying various studies carried out by American Association of Clinical Oncology from October 2005 to September 2014, the last updated version respecting the use of this factor was released in July 2015. It is also worth noting that Hematopoietic colony-stimulating factors (CSFs) can be used as preventers when the risk of fever and neutropenia is more than 20% and no treatment protocol can be replaced for this factor.

Furthermore, in primary prevention, when considering the patient's condition (age, nature of the disease, chemotherapy regimen required, etc.), the risk of high fever, and neutropenia is high, application of this factor in Dose-Dense Chemotherapy regimens is required. The so-called application has to be limited to the clinical trials that are designed appropriately and are backed by useful and effective information and findings. Applying this this factor for patients exposed to deadly radiation of whole body radiotherapy has been approved. [33]

Reducing the severity of adverse effects of chemotherapy, CSF is one of the available drugs in Iran that has made possible the using new methods and treatment programs in this country, and is presently considered as an inevitable part of modern methods in post-chemotherapy treatment. Until recently, the only existing form of G-CSF in Iran was Neupogen produced by Roche Co. This drug, due to the high incidence of cancer, the extent of use, and the high price imposed a high cost burdens on patients and their families, insurance organization, as well as to the Ministry of Health. Currently, G-CSF is either being provided by several local companies in the market or is being studied (clinical and quality control related) for permission to enter the market. Tinageast is made by AryaTinaGene Company, and all quality controls have been applied on its production and the findings have confirmed its high quality wich is comparable to the reference product (Neupogen). The aim of the present study is to approve the equal efficacy of this drug with the reference product in terms of incidence and severity of neutropenia incidence of fever and neutropenia after chemotherapy in cancer patients.

Innovation of the Project:

The present project leads to the use of an Iranian biosimilar drug for 25 percent below brand reference price and saves a large amount of money for the country.

Review of literature

In a surve carried out using international databases, with the exception of the results of Phase 1 and 2 clinical trials of granulocyte stimulating factor, which was conducted between 1989 and 1998, phase 3 clinical trials on patients with cancer almost began in 1995. It is quite natural that the early trials were all in non- randomized or open-label form. However, through time, some more standard designs of these randomized, double-blind trials improved (using a control group, with or without placebo or not).

One of the first randomized, double-blind clinical trials was performed on the elderly patients with acute myeloid leukemia by Gogwin et al (1998) [34]. 255 patients aged 55 years or older with tumors were randomized into two groups: in one of them, chemotherapy protocol was applied with a placebo (control group), and, in another group, the same protocol was used with G-CSF at the dose of 400 mg per square meter of body surface, infused intravenously, within 30 minutes once a day for the duration of chemotherapy treatment. Regarding the index of compete response, the two groups were not statistically dirrerent (50% in the placebo group and 41% in G-CSF group). In addition, in terms of Overall Survival rate, the two groups had the same status of the indicator of therapeutic response, which was not statistically significant significant (9 months in the placebo group versus 6 months in the group G-CSF. However, the time required for the neutrophils to be improved in the G-CSF group was 15 percent shorter than the placebo group, determining the difference to be statistically significant. Although the use of G-CSF did not reduce neither the overall incidence of infections nor the incidence of fatal infection, the duration of the infection and the need for antibiotics in the G-CSF group was lower than those of the placebo group, and this difference was statistically significant.

Thatcher et al. (2000) [35] evaluated the effect of G-CSF (Lenograstim) to improve survival in patients with small cell lung cancer (small cell lung cancer) in a multicenter clinical trial conducted in the UK Medical Research Council. In this trial, 403 patients diagnosed with this cancer were randomly assigned to either a control group (group C) and G-CSF (group G). patients in Group C were treated with three-drug chemotherapy protocol (doxorubicin, cyclophosphamide and

Etoposide) every three weeks for 6 cycles, and those in Group G were treated with the same protocol but with a 50% higher dose every two weeks plus Lenograstim.

The findings of this multicenter study showed that the response rate in Group G was about 40% and 28% Group C, which was statistically significant. The rate of survival was higher in group G in comparison with group C (hazard ratio was 0.80, with 95% confidence interval 0.65 to 0.99).

Carbonero et al. (2001) [36] evaluated the effect of G-CSF treatment in a multicenter clinical trial on cancer patients with fever and neutropenia risk. 210 patients with solid tumors ,suffering from fever and neutropenia grade 4 fter conventional chemotherapy protocol had been administered, were divided randomly into two groups: one with antibiotics plus Ceftazidime Amikacine without G-CSF (control) and the other with G- CSF at the dose of 5 mg/kg per day (intervention group). The mean duration of grade 4 neutropenia in G-CSF group was 2 days and for the the control group was 3 days ($p < 0.05$), in addition, the mean duration of hospitalization in G-CSF group was about about 5 days compared to 6 days in the control group; the difference was also statistically significant.

Giglio et al. (2008) [37], in a phase 3 clinical trial, studied the superiority of XM02 to placebo and its equality in effectiveness compared with Neupogen in reducing the incidence of severe neutropenia, fever and neutropenia in patients with breast cancer receiving doxorubicin/docetaxel regime.
348 patients in 10 countries and 52 centers in 3 groups with the ratio of 1.2.2: the placebo group (n = 72), Neupogen group (n=136), and XM02 gruop (n=140). Filgrastim was injected at the dose of 5μg / kg / day starting one day after chemotherapy administration for a duration of at least 5 days and a maximum of 14 days. The primary outcome variable was the duration of severe neutropenia in the first cycle that , in both XM02 (1.1 days) and Neupogen group(1.1 days), was almost one third of the placebo group (3.9 days). Difference of the variable between the two treatment groups was 0/028 days, which was in the same range.

The incidence of fever and neutropenia in the first cycle were the same in Neupogen group (12.5%) and XM02 group (12.1%), and it was almost one third of the placebo group (36.1). Absolute neutrophil count fluctuated similarly in both treatment groups. To evaluate the safety, the adverse

effects of the three groups were studied; bone pain and asthenia were reported with a higher frequency, with similar rate of occurrence in 2 groups.

Patients in the placebo group after completion of the first cycle of chemotherapy drug, XM02 received and the amount of severe neutropenia, neutrophil count decrease mean time to recovery of neutrophils status as secondary outcome variables were compared in all cases with drug-friendly approved reference drug.

Waller et al. (2010) [38], in a double-blind, multi-center ,phase 3 clinical trial, studied Nivestim produced by Hospira against Filgrastim, an Amgene product, in breast cancer patients receiving chemotherapy in Europe. 279 patients entered this study and were categorized into two groups with the ratio of 1.2: Nivestim Group (184 people) and Neupogen group (95 cases). Filgrastim was given by subcutaneous injection at the dose of 5μg / kg / day since the second day to a maximum of 14 days per cycle. The primary outcome variable was the duration of severe neutropenia in the first cycle– 1.3 days in Neupogen Group and 1.6 days in Nivestim group (95%: CI); it approved that these two drugs were bioequivalent. The secondary outcome variables of the study were the duration of severe neutropenia in the second and third cycle, the duration of neutropenia improvement, and the incidence of febrile neutropenia in the first to third.

No notable changes in laboratory parameters were observed in group 2. Studying fever and neutropenia showed approximately the same results: 12.57% for the Nivestim group and 12.63% for the Neupogen group. Furthermore, respecting adverse effects, a similar percentage was observed (86.9% vs. 84.2%) in both Nivestim and Neupogen groups, 86.9% versus 84.2%, respectively.

Regarding hospitalization rate due to fever and neutropenia, no statistically significant difference between the two groups was seen. Since no neutralized antibodies against the drug could be found in any patient, in 2010 this drug received acceptance by EMA, like all indications of reference Filgrastim.

Beksac et al (2011) [39] in a multicenter, open-label clinical trial conducted based on Turkey leukemia study, 260 patients with acute myeloid leukemia having less than 500 neutrophils per cubic mm were randomly divided into two groups: one group (control group) received conventional chemotherapy or induction chemotherapy and the other group (intervention group)

received Neupogen or Filgrastim produced by Roche. In coth groups, the baseline variables were similar in two groups. The total response rate between the two groups showed no significant difference. Although the median duration of hospitalization in the Neupogen group was four days less than that of the control group (31 days versus 35 days), this difference was not statistically significant. Weekly patient follow-up of showed the same white blood cell count in both groups at the end of the first week (average 700 per cubic millimeter of blood). However, at the end of the second, third, and fourth week, this index in Neupogen group was better than the control group. In the last follow-up or the fourth week, the average difference in white blood cell count between the two groups was statistically significant (3200 per cubic millimeter in Neupogen group versus 1800 in the control group).

Ruiz et al (2011) [40], in a phase four clinical, non-randomized, and open-label trial in Cuba studied the efficacy and safety of leukoCIM in neutropenic patients following chemotherapy.
Of 47 patients, 95 who had experienced neutropenic period during treatment entered the study (retrospective study). Patients were categorized into two groups based on the Filgrastim received– prophylactic or therapeutic. Defined as the main variable was the delay or non-delay of the next cycle of chemotherapy response. Statistically, there were no relationship between the main variable response and the type of group (prophylactic or therapeutic (80.7% and 84.2%).

The average absolute neutrophil count at the beginning and at the end of the cycle were calculated 1.490 and 5.51 ml, respectively, in addition, the maximum time to improve neutrophils were measured one week. Thus, 82.1% of patients received the next cycle of chemotherapy without delay. In this regard, this product was reported to be as effective as other granulocyte growth stimulants. Product Safety was also assessed by examining the adverse effects; the most severe reported adverse reactions were fever (11.22%) and bone pain (11.22%), taken into account as common side effects of Filgrastim.

In a non-randomized, multi-center study in Japan, Sagara et al. (2013) [41] inquired into the safety and effectiveness of Fsk0808 (Filgrastim) in patients with breast cancer. During the study, 104 patients within 6 cycles of chemotherapy (of 413 cycles) entered the study. The primary outcome variable was the mean duration of grade 3 neutropenia in the second cycle , calculated as 2.2 (SD:105) (CI :97 %, unilateral 2.2 days).

Defined as the secondary outcome variable was the incidence of fever and neutropenia and tracking Anti GCSF (antibody), being reported as 34.6% and 0, respectively. In this study, the incidence of fever and neutropenia was reported to be relatively high compared to previous similar studies. Although the reason was not clear, it was believed to be due to drug use to treat (rather than prevent) fever and neutropenia. Mostly recorded adverse effects were back pain (60.6%) and bone pain (9.6%).

The findings of this study showed that this drug was well tolerated by patients, resulting effectively in improvement of neutrophils in patients with breast cancer undergoing chemotherapy.
In a study, Blackwell et al, (2015) [42] compared EP2006 and Neupogen in patients with breast cancer receiving chemotherapy regimen for prevention of neutropenia resulting from it. A phase III clinical, double-blind and randomized trial was conducted on 218 patients at 25 centers. Patients were categorized in 4 groups at the ratio of 1.1.1.1 as well as in two intermittent, non-periodic groups. Alternating cycles of chemotherapy in the patients in the group receiving Neupogen or EP2006 is taken in alternating groups in each cycle change. Filgrastim was administered since the second day each cycle for up to 14 days at the dose of 5 μg / kg / day

The duration of severe neutropenia (primary variable consequences of drug efficacy) was reported to be 1.02 ± 1.20 and 1.11 ± 1.17 in Neupogen group (including 105 patients) and EP2006 groups (including 101 patients), respectively (CI:0.97), showing no significant difference.

Drug safetywas evaluated by studying the adverse effects and Anti GCSF antibody production. The adverse effects, resulting probably from receiving Filgrastim, were reported to be similar between Neupogen (19.6%) and EP2006 group (20.6%) in the first cycle. In addition, antibody production rate was zero. This drug was approved by the U.S. Food and Drug Administration in March 2015 and was introduced as the first biosimilar Neupogen in the U.S.[43].

Studies published in Iran

Among studies carried out in Iran, two clinical trial reports were found. In a randomized, double-blind, and cross-over d trial, Moafi et al. [44] made a comparioson between the therapeutic and adverse effects of the granulocyte stimulating growth factor produced by a local manufacturing

company called PD-Grastim with those of Neupogen produced by Amgen (the reference treatment) in children aged 1 to 15 with variety of cancers. In each treatment group, 30 eligible children received either PD-Grastim or Neupogen for 4 days at the end of their standard chemotherapy treatment. Since the trial is a crossover one, it was followed by a different chemotherepy for each chiledand in the next cycle of chemotherapy.

The average white blood cell count, neutrophil percentage, and absolute number of neutrophils in both groups were alsmost the same and there was no statistically significant difference. Hospitalization occured due to fever and severe neutropenia in 3 patients in Neupogen group and in 4 patients in PD-Grastim group, indicating no statistically significant difference between these two groups. Nothing has been mentioned about the wash-out period (to clean up the effects of the first drug used), and the beginning of the next treatment. This is associated with the general principle of cleansing side effects of the drug for a period wich is 7 times longer the half-life of the drug [45], regarding the fact that that Filgrastim half-life is T1 / 2 = 3-4 h [46].[

In a similar study on 8 children aged under 16 with neuroblastoma in Bahrami Children's Hospital, Ehsani et al. [47] measured the differences between the effectiveness and side effects of the Filgrastim produced by Amgen Filgrastim (in the U.S.) with those of its Iranian biosimilar i.e. PD - Grastim.

Other studeis registered in clinical trials registry of Iran.

The implementation methods of these trials are briefly described as follows [48].

In a cross over study, Dr. Youssefian et al. compared the effect of Filgrastim and Peg Filgrastim on the treatment of 33 neutropenic patients under 16 years of age. As the consequence of the study variables, absolute neutrophil count is evaluated one week after adverse effects of drug intervention within 7 days.

In another study, dr. Homaeizxcc CACC to compare the effectiveness and side effects Filgrastim produced within the country and have Nyvpvzhn. 168 patients with breast cancer were divided into two groups, and the study was designed in parallel. Drug side effects, duration of neutropenia and neutropenic frequency between the two groups were evaluated.

Dr. Razavi et al. studied the efficacy, safety, and tolerability of the Filgrastim and Peg Filgrastim produced domestically in Iran compared with Neupogen in the prevention of neutropenia after chemotherapy. 210 patients with breast cancer were divided randomly into 3 equal groups. Various parameters including the number of days of severe neutropenia infection, febrile neutropenia, abnormal laboratory tests, adverse effects, etc. were evaluated.

Dr. Salimi et al. compared the efficacy and adverse effects of Filgrastim produced domestically in Iran with those of reference product in patients with gastric cancer. 60 patients entered the study; white blood cells, hemoglobin, platelets after treatment with the drug were determined as the primary variable consequences.

Purposes and hypotheses

General Purposes

- Comparing therapeutic and prophylactic effects of Neupogen and Tinagrast on fever and neutropenia following chemotherapy in patients with breast cancer

Specific purposes

- Comparing therapeutic and prophylactic effects of Neupogen and Tinagrast based on incidence of severe neutropenia in patients with breast cancer
- Comparing therapeutic and prophylactic effects of Neupogen and Tinagrast based on febrile neutropenia incidence index in patients with breast cancer
- Comparing therapeutic and prophylactic effects of Neupogen and Tinagrast based on other hematological parameters (white blood cell and neutrophil count in patients with breast cancer
- Comparing therapeutic and prophylactic effects of Neupogen and Tinagrast based on febrile neutropenia incidence index in patients with breast cancer, according to background variables such as age, severity of illness, etc.
- Comparing therapeutic and prophylactic effects of Neupogen and Tinagrast Compare the efficacy of prophylaxis with Neupogen Tinageast febrile neutropenia incidence index (Febrile Neutropenia) in patients with breast cancer based on the baseline variables such as age, severity of illness, etc.
- Comparing therapeutic and prophylactic effects of Neupogen and Tinagrast Compare the efficacy of prophylaxis with Neupogen Tinageast based on other hematological parameters

(white blood cell and neutrophil count in patients with breast cancer concerning the baseline variables such as age, severity of illness, etc.

- Comparing the occurrence of severe adverse effects of Tinageast with Neupogen

- Comparing the occurrence of severe adverse effects of Tinageast with Neupogen

The severe side effects in the two treatment groups Tinageast with Neupogen based on the baseline variables such as age, severity of illness, etc.

Hypotheses

- The therapeutic and prophylactic effects of Neupogen and Tinagrast do not differ based on primary and secondary primary variable consequence.
- Occurrence of severe and dangerous adverse effects of Tinageast do not differ with those of Neupogen.

Research Method

The present study has been designed as a randomized, double-blind, multicenter, parallel trial. Allocation ratio is 1:1.

Inclusion Criteria

1. Clinical phase: breast cancer stage 2 and higher
2. Age: over 18 years
3. ECOG PS = 0,1,2[18]
4. Cancer history: No prior cancer
5. Receiving chemotherapy: no prior chemotherapy
6. Adequate bone marrow activity: defined as follows:

   leukocytes>/=3,000/µl

   absolute neutrophil count >/=1,500/µl

hemoglobin>/=8.0g/dl

platelets>/=100,000/µl

7. Left ventricular ejection fraction ≥ 50% (using echocardiography)
8. Proper liver function

alanine and aspartate aminotransferases <2.5 × upper limit of normal

9. Liver failure

serum creatinine must be < 1.5 mg/dl

Exclusion criteria:

1. Age: over 65 years and less below 18 years
2. Coincidence with disorders or other severe disease
3. ECOG PS = 3,4
4. Pregnancy or breast-feeding
5. Left ventricular ejection fraction: weak or mixed
6. Uncontrolled seizures or poor state of mind
7. Active infectious diseases
8. Cancer history
9. Chemotherapy History
10. Undergoing major surgery (except for the current disease) during the past 4 weeks

Research Population and Location:

Subjects were breast cancer patients who were referred to the following Oncology Centers to receive chemotherapy: Panje Azar hospital under the supervision of Golestan University of Medical Sciences, Imam Hossein hospital under the supervision of Shahid Beheshti University of Medical Sciences, FayazBakhsh Hospital under the supervision of and Tehran University of Medical Sciences, and Ayatollah Khansari Hospital under the supervision of Arak University of Medical Sciences.

Time of the study:

From June 2003 to November 2015

Intervention:

In this clinical trial intervention is the granulocyte growth stimulating factor.

Tinagrast: a granulocyte growth stimulating factor, a recombinant produced by AryaTinaGene Company, that is available in pre-filled syringes with the dose of 300 micrograms in 0.5 ml; it was applied subcutaneously at the dose of 5 micrograms per kg per day for 6 days in the intervention group.

Neupogen: a granulocyte growth stimulating factor, a recombinant produced by Amgen Company, that is available in pre-filled syringes with the dose of 300 micrograms in 0.5 ml; it was applied subcutaneously at the dose of 5 micrograms per kg per day 24 hours after chemotherapy for 6 days in the intervention group.

The outcomes of the study and their definition:

The primary outcome was defined as follows:

The incidence of neutropenia in patients during chemotherapy that was classified through blood test follows:

- Grade zero 1500 <ANC
- Grade one 1000<ANC <1500
- Grade two 500 <ANC <1000 (mild neutropenia)
- Grade three 200 <ANC <500 (average neutropenia)
- Grade four 200 <ANC (severe neutropenia)

Neutrophil count based on blood samples and cell count measurements on the ninth day of each chemotherapy cycle is recorded in the table. (Table 3)

Secondary outcomes were defined as follows:

1.Incidence of neutropenic fever (fever over 38 ° C for at least an hour, as well as neutropenia less than 500 per cubic millimeter in the day that the fever occurs) that was evaluated using blood tests and classified as follows:

- Grade zero without fever
- Grade one 38-39
- Grade two 39.1-40
- Grade three 40 <for less than 24 hours
- Grade four 40 <for more than 24 hours

2. Incidence of neutropenia without fever; its severity was classified as a follows:

•Grade zero without fever

• Grade one 38-39

•Grade two 39.1-40

•Grade three 40 <for less than 24 hours

• Grade four 40 <for more than 24 hours

3. Incidence of blood disorders such as anemia; its severity was evaluated using blood tests and classified as follows:

•Grade zero Hb = Nl

•Grade one 11 <= Hb <12

•Grade two 10 <= Hb <11

•Grade three 9 <= Hb <10

•Grade four 9 <Hb

4. Incidence of thrombocytopenia and bleeding; its severity was evaluated using blood tests and classified as follows:

•Grade zero normal number of platelets

•Grade one a mild thrombocytopenia, not requiring transfusion

•Grade two medium thrombocytopenia, not requiring transfusion

•Grade three severe thrombocytopenia, not requiring transfusion

•Grade four life-threatening thrombocytopenia, requiring urgent transfusion

5. Incidence of nausea; its severity is recorded through the history of the patient and is classified as follows:

•Grade zero no symptoms

•Grade one normal oral feeding

•Grade two decrease oral intake

•Grade three no oral feeding, infusion

• Grade four no oral feeding, requiring urgent infusion

6. Incidence of vomiting; its severity is recorded through the history of the patient and is classified as follows

• Grade zero no symptoms
• Grade one one episode in 24 hours
• Grade two two to five episodes in 24 hours without treatment
• Grade three six or more episodes, requiring infusion
• Grade four hemodynamic collapse, requiring urgent infusion

7. Incidence of diarrhea; its severity is recorded through the history of the patient and is classified as follows:

•Grade zero no symptoms
•Grade one less than 4 bowel movements in a 24-hour period
•Grade two 4 to 6 bowel movements in a 24-hour period
• Grade three 7 or more bowel movements in a 24-hour period, requiring infusion
• Grade four hemodynamic collapse, requiring urgent infusion

8. Risk or incidence of bone pain associated with the growth factor granulocyte; its intensity through the history of the patient and floor were classified as follows.

•Grade zero without pain
• Grade one pain without the need for tranqualizers
•Grade two pain control with tranquilizers
•Grade three pain control with tranquilizers but recurring
• Grade four uncontrolled pain

9. Incidence or occurrence of muscular pain associated with granulocyte growth factor; its severity is recorded through the history of the patient and is classified as follows:

•Grade zero without pain
• Grade one pain without the need for tranqualizers
•Grade two pain control with tranquilizers
•Grade three pain control with tranquilizers but recurring
• Grade four uncontrolled pain

10. Local reactions at Filgrastim injection site; its severity is recorded according to the size of the response obtained via physician`s observation and classified as follows:

•Grade zero without reaction
•Grade one 5 -5.2 cm

- •Grade two 10-1.5 cm
- •Grade three 10 cm < reaction size
- • Grade four Necrosis-centimeter of the site or exfoliative dermatitis

Secondary outcomes on the tenth day of each chemotherapy cycle was also evaluated by the specialist and was recorded in Table 3. Patients were linked directly to the treating specialists; in case of complication occurrence at any time during the study, reports were necessary to transfer them. The necessary decisions was then made to whether continue or discontinue the drug treatment by the physician. The specialists were also provided with ADR forms to make records in case of incidence of serious side effects; in addition, the pharmacovigilance department of the supportive company was informed.

Trial sample size:

Since this trial, based on the objectives of the study, is designed as a Equivalence Trial, the sample size equations relating to the trials were applied. The following equation was extracted from reference [49].

$$n = 2(Z_{\alpha} + Z_{\beta})^2 \Delta^2$$

In this formula, Delta is the standard deviation difference between the two groups and n is the sample size in each group.

However, according to the reference [50], using table 3 in this article, mean and standard deviation (duration of grade 4 neutropenia) were 1.1 ± 0.9 and 1.2 ± 1.1 in the two groups, respectively. Standard deviation of the difference between the two groups was computed 1.63. When these generated numbers are placed in the above-mentioned formula, the sample size in each group was determined about 45 people. Considering the 10% loss to follow-up, the total number became 50 patients in each group and 100 in total, regarding the parallel design of the.

Randomization Method:

In this clinical trial, randomization of treatment allocation was Block Balanced Randomization method using quaternary blocks A and B. Random sequence generation of treatment group using this method is as follows:

Obviously, using the two above-mentioned letters, six blocks of four can be generated: AABB, BBAA, ABAB, BABA, ABBA and BAAB. Each of these six blocks, from right to left, is assigned a number from 1 to 6. In order to generate codes A and B in random order, 25 random numbers (obtained by dividing the number of the sample size or 100 by the number of letters in each block i.e. 4) from 1 to 6 (using table of random numbers or statistical software) can be chosen. Each numbers 1-6 represents the corresponding block of four; therefore, 25 blocks of four are generated from right to left, determining the treatment group orders. Obviously, 25 blocks of four produces 100 sequences, based on which the patients, ordered by inclusion, received the necessary treatment. Regarding the parallel design of the, all patients received treatment A or B.

The list of blocks and treatment groups sequence was prepared by statistical consultant of the project; this list will be confidential to them. Before the beginning of the implementation phase of the study, cards on which letters A or B are printed were produced and were put in envelopes. Card sorting was performed by statistical consultant of the project in accordance with the block sequence. Cooperating specialists in the study evaluated the treatment process and the outcomes from the time the study began. They assigned one of the above-mentioned envelops, from right to left, to each patient as each patient entered the study. In addition, the person injecting the drug (the nurse) also was given the growth factors delivered according to the codes (A and B). This person was the only one who is aware of the nature of the codes fro he is assumed responsibility for covering the needle with the white paper and did not interrupt the registration and evaluation of outcomes.

Blinding and its implementation

This clinical trial was a double-blinding one. In other words, both patients and doctors responsible for evaluating the outcomes, were not aware neither of the nature of growth factor injected into patients nor of the true nature of A and B codes. This is because the shape of Tinageast and Neupogen pre-filled syringes, as well as their dose, duration of use, and prescription method were quite similar. In addition, the nurse, to prevent syringe label to seen by the patient before the injection, syringes were covered with white wrapper.

At the beginning of the study period, patients, after being randomly assigned to two groups i.e. A and B, were treated with 4 cycles of TAC chemotherapy (three weeks per cycle) (Table 1).

Project implementation method

In the present trial, the goup receiving Tinagrast was named A, and the other group (control goup) receiving Neupogen was called B.

The distribution of patients in the two treatment groups (of four treatment phases) can be seen in Table 2. For each patient at the beginning of treatment, baseline CBC test was carried out. Then, chemotherapy began (the first day); 24 hours after chemotherapy (day 2), Filgrastim was injected daily at the dose of five micrograms per kg body weight (one syringe) up to the seventh-day (6 days). Day 8 was a rest day and on the ninth day, CBC test was carried out. Those patients with neutrophils count less than 500, at the discretion of the treating specialist, either were given the growth factor for more days (at most 5 days) or were put under medical supervision (receiving no Filgrastim) . Tests and Filgrastim injection procedure were repeated until the fourth cycle of chemotherapy.

Chemotherapy was administered on days 1, 22 ±1, 43 ±1, and 64 ±1 ; in addition, patients were screened on days 9 ± 1, 31 ± 1, 52 ±1, and 73 ±1. (Table 2)

For all patients (in both groups A and B), blood samples to determine the WBC, ANC, Platelet Hemoglobin once before chemotherapy and after nine days of treatment evaluated Qrargrftnd. Patients were evaluated per cycle two times (the first day and the ninth day) by radiotherapy specialist assessed.
No such symptoms as fever[19], diarrhea, nausea and vomiting, musculoskeletal pain, local reactions at the injection site nor any other side effects such as a low blood cell count (in Table 3 and on the ninth day of each cycle) were recorded by the treating specialist.

TREATMENT: Drug	Dose	BCCA Administration Guideline
Doxorubicin (Adriamycin)	50 mg/m²	IV push
Cyclophosphamide	500 mg/m²	IV in 100-250 mL NS or D5W over 20-60 minutes

Docetaxel(Taxoter)	75 mg/m2	IV in 250 ml NS over 60 min (use non-PVC equipment)
Filgrastim(G-CSF)	5mcg/kg/day(1 syringe) Day:2-7	SC

Table 1: TAC Chemotherapy protocol specifications

Table 2: Schedule

FLOW CHART

Visit No	1	2	3	4	5	6	7	8
Day	1	9±1	22±1	31±1	43±1	52±1	64±1	73±1
Activity	Screening /Treatment	visit	Treatment	visit	treatment	visit	Treatment	visit
Consent	X							
History	X							
Pathology	X (a)							
Pathological and Clinical Staging (b)	X							
Drug dispensing	X		X		X		X	
Blood sample (C)	X	X	X	X	X	X	X	X
Whole body bone scan	X							
CT scan of Thorax and abdomen	X							
Concomitant Medication	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X

a) One pathological result is enough – the historic data will be used.
b) Size of tumor according to greatest diameter, Extension and Number of lesions (T stage), their location, presence of distant metastases, and regional lymphadenopathy.
c) Blood samples of screening day, days 1,9,22,31,43,52,64and 73 are used for safety (CBC)

Table 3: Outcomes of the study

Adverse Events

Adverse Event	0	Grade 1	Grade 2	Grade 3	Grade 4
Absolut Neutrophil count	NL ○ 1500<ANC	ANC 1000-1500 ○	ANC 500-1000 ○	ANC 200-500 ○	ANC <200 ○
WBC/mm³	NL ○ 3000<	2000-3000 ○	1500-2000 ○	1000-1500 ○	<1000 ○
Platelet	NL ○ 150000<	150000 ○ ≤ 75000	75000 ○ ≤ 50000	50000 ○ ≤ 10000	< 10000 ○
Haemoglobin	NL ○	11 ≥ Hb > 12 ○	10 ≥ Hb > 11 ○	9 ≥ Hb > 10 ○	9 < Hb ○
Thrombocyto penia & bleeding	None ○	Mild no need to transfusion ○	Moderate no need to transfusion ○	Sever need to transfusion ○	Life treating need emergency transfussion ○
Fever & Neutropenia	None ○	38° - 39° C ○	39°.1-40° C ○	T>40° C less than 24h ○	T>40° C more than 24h ○
Fever without Neutropenia	None ○	38° - 39° C ○	39°.1-40° C ○	T>40° C less than 24h ○	T>40°C more than 24h ○
Nausea	None ○	Normal oral feeding ○	Oral feeding decreased ○	No oral feeding need infusion ○	No oral feeding need emergency infusion emergency ○
Vomiting	None ○	1 episode in24h ○	2-5 episode in24h no treatment ○	6 and More episode need infusion therapy ○	Hemodynamic claps need emergency infusion therapy ○
Diarrhea	None ○	Less than 4 times stools in 24h ○	4-6 times stools in 24h ○	7 times more stools in 24 h need infusion therapy ○	Hemodynamic claps need emergency infusion therapy ○
Bone pain	None ○	No need sedative ○	Controlled with sedative ○	controlled with sedative but repeatable ○	Do not controlled with sedative ○
Muscle pain	None ○	No need sedative ○	Controlled with sedative ○	controlled with sedative but repeatable ○	Do not controlled with sedative ○
side of injection Erythema/ Redness Reaction	None ○	2.5 – 5 cm ○	5.1 – 10 cm ○	10cm < ○	Necrosis or exfoliative dermatitis ○

Data collection and sampling method

All patients` information, including age, weight, height, staging, testing, and primary and secondary outcomes, etc. has been recorded on Case Report Form (CRF) by treating specialists.

Methods of statistical analysis

In this clinical trial, after reviewing every single Case Report Forms (CRF) or subjects of study, in order to ensure that all data–those being assessed– has been included and verified; the data have been into version 16 of SPSS. The verification of these variables and the assessment of their distribution were performed using the previously mentioned software. Statistical analysis was performed using the STATA version 12. Assessment of the normal distribution of quantitative variables was performed using the Kolmogorov-Smirnov test. Variables were described using tables and error bar (to compare the distribution of quantitative variables in the baseline period and four follow-up periods).

Background variables were compared in the baseline phase using t-test or chi-square test. In case of normal distribution failure of each quantitative variable, nonparametric test (Mann-Whitney U test) was used.

To assess the quantitative variables (blood cell indices) during follow-up periods and in two treatment groups repeated measures ANOVA tests was used. All multiple pairwise comparisons were performed using Scheffé's method (this method correct the increase in Type I error resulting from Multiple Comparison test).

In order for making a comparison among the efficacies of the two drugs in four periods, the indicator of treatment success was defined first. This indicator is derived from dividing the total population(with normal neutrophil count) at any period by all subjects in the treatment group. A comparison was made between the two groups regarding this indicator; it was done using Risk Ratio (dividing two indicators of treatment success by Neupogen treatment group to the same index in the same period in Tinagrast), Risk Difference (difference between the two indices referred treated successfully at any time), and a 95% confidence interval.

The above-mentioned calculations were performed in the two treatment groups. In addition, these two groups were compared using Per Protocol approach analysis, Intention-to-treat analysis (ITT), as well as different scenarios for the replacement of missing data in different follow-up periods. All assumptions were tested bilaterally and the significance level of all Statistical tests was set to 0.05.

Ethical Considerations

Given the nature of this study and the need to obtain biological samples (blood or serum), written informed consentforms were developed prior to the beginning of the study by executives. Patients were informed and were asked to carefully read and sign the consent forms. In addition, patients could be excluded from the study any time they intended. Participants were ensured about the confidentiality of information to the executives.

The study protocol was approved by research ethics committee of Glstanba University of Medical Sciences (code 77392032906) and by Arak university of Medical Sciences (Code IR.ARAKMU.REC.1394.180).

Clinical Studies

This clinical trial was recorded in Iranian database of clinical studies under RCT2013062613776N1.

Study Limitations

The high cost of the materials and facilities needed for the study as well as the large number of specimens has brought about some limitations to the study; preparation of reference product was a burden at the beginning of the study. The large number of the specimens along with following up the treatment at four stages added to the difficulty of carrying out this study, and the study progressed behind the schedule. Furthermore, regarding the changes in preferred regimen in NCCN guideline (2014), TAC chemotherapy regimen changed from a “preferred” regimen to “other regimen” after surgery. Therefore, to comply with the research protocol and international updated cancer guideline, patients selected for treatment were not preferably among those not undertaking surgery before receiving chemotherapy. In this way, both research protocol and international standards were considered. Inevitably, these barriers led to delayed patient choosing.

Findings

Clinical Trial Analysis of Tinagrast

a) Distribution of background variables and important variables in the baseline phase (Baseline) or before the beginning of the treatment based on treatment groups according to CONSORT standard:

It is recommended that Table 1 in each clinical trial be allocated to compare the distribution of background variables with other factors associated with response to treatment. The table, in addition to being one of the most effective components on drawing conclusion in this study, indirectly shows whether or not the performance of Random Allocation in the allocation of two or more treatments to patients has been successful.

It should be also noted that the conclusion of this table has not been based on the significance of the difference was, but on mean differenced or important frequencies. Taking the previously-mentioned explanations into account, Table 1 is described as follows:
In Table 1, the distribution of the number of patients into two treatment groups has been outlined based on the separation of background variables and other variables affecting the treatment.

Table 1: Distribution of patients treated with these Neupogen and Tinagrast based on baseline variables and other important variables in decision-making for therapeutic response in patients

variables	Treatment with NEUPOGEN (n=49)	Treatment with Tinagrast (n=43)	P value
Patient`s age	10.1 ± 42.8	11.5± 45.9	0.17†
Patient`s weight	13.9 ± 72.0	10.8 ± 68.0	0.13†

Patient`s stage of cancer Stage 2 Stage 3 Stage 4	)55.6 (25)37.8 (17)6.6 (3	(۵۲.۴)22 ۱۱ (۲۶.۲) ۹ (۲۱.۴)	۰.۱۱$
White blood cells❋	۶۸۵۱ ± ۱۷۸۸	۷۰۴۴ ± ۲۱۸۰	†۰.۶۴
Absolute neutrophil count❋	۴۱۷۸ ± ۱۳۷۸	۴۳۹۳ ± ۱۶۶۷	†۰.۴۹
Red blood cells *(×۱۰۰۰)	۴۳۴۳ ± ۴۳۵	۴۳۴۶ ± ۸۲۲	۰.۳۸&
Platelet count*(×۱۰۰۰)	۲۷۸.۳ ± ۸۴.۰	۳۱۵.۴ ± ۱۱۹.۵	†۰.۰۹

❋A few patients were not determined at baseline staging position (4 patients in Neupogen group and 1 in Tinagrast Group).

In addition, in the case of CBC indicators, only one person in the Neupogen group lacked these clinical tests.

†:According to independent t test

$: Based on the Chi square,

&: Mann-Whitney test (due to lack of normal distribution of red blood cells (see Figure 3).

Description of Table 1:

In any clinical trial, Table 1 has been one of the most important components in these studies; distribution of background variables as well as variables influencing the response to treatment may affect the final conclusions in any trial. For instance, the effects observed at the end of treatment cannot be definitely attributed to the administration of the drug in the trial. This happens if the distribution of a factor affecting the response to treatment (e.g. the absolute number of neutrophils in this study) at baseline stage or before the beginning of the treatment is significantly different in two treatment groups (as mentioned earlier, the

difference was not considered statistically significant and, in fact, clinically relevant difference is considered). For example, in the present study, can we attribute the improvement of the same blood index (i.e., absolute neutrophil count) to the efficacy of Tinagrast at the end of the fourth treatment cycle, if the average neutrophil count per cubic millimeter in the two Neupogen and Tinagrast groups at baseline stage is 2500 and 4500, respectively?

On the other hand, another application of the table is to clarify how the random allocation works. There exist various methods of random allocation of two or more therapies in clinical trials. From a practical point of view and regardless of determining which random allocation methods is theoretically preferable, the uniform distribution of background variables and other variables affecting response to treatment shows the proper functioning of this phenomenon in this clinical trial.

As a result, carefully reviewing the variables described in Table 1, it can be found that the random assignment of the method in this trial functions properly. Even if the differences in some variable are not precisely pointed to, it can be realized that the differences are mainly in favor of Neupogen group (control group in this trial) but against Tinagrast group (treatment group in this trial). This is due to the fact that in Tinagrast group the percentage of stage 4 cancer patients was higher than that of the control group (about 15% higher for stage 4 cancer patients). Furthermore, in terms of age and weight as two background variables of at baseline stage, the average age of the patients in the Neupogen group was three years younger than that of patients in the Tinagrast group; the average weight of the Neupogen goup was 4 kg more than the average weight in other groups . It should be noted that in Table 1 none of the differences were statistically significant.

Regarding the distribution of Blood cell indices or complete blood count (CBC), as important variables that can affect the response to treatment, the situation was slightly different with the distribution of background variables in two groups. In other words, concerning these four variables, distribution of CBC variables is in favor of Tinagrast since, in the baseline phase, the average number of these cells in this group was more than that of in Neupogen group. It should be noted that, in further analyses, the effect of these differences were entered in statistical models so that the final result is unbiased.

b) Statistical distribution assessment (normality) of hematological parameters in the baseline phase based on the two treatment groups:

Parametric statistical tests (more importantly T tests as well as those like ANOVA) include relatively similar assumptions. These assumptions are as follows:

1- Quantitative variables being studied in two or more treatment groups have a normal distribution.

2- Variance (or standard deviation) of this quantitative variable is equal in two or more treatment.

Normality assessment of white blood cells in the baseline phase in the two treatment groups can be observed in Diagram 1.

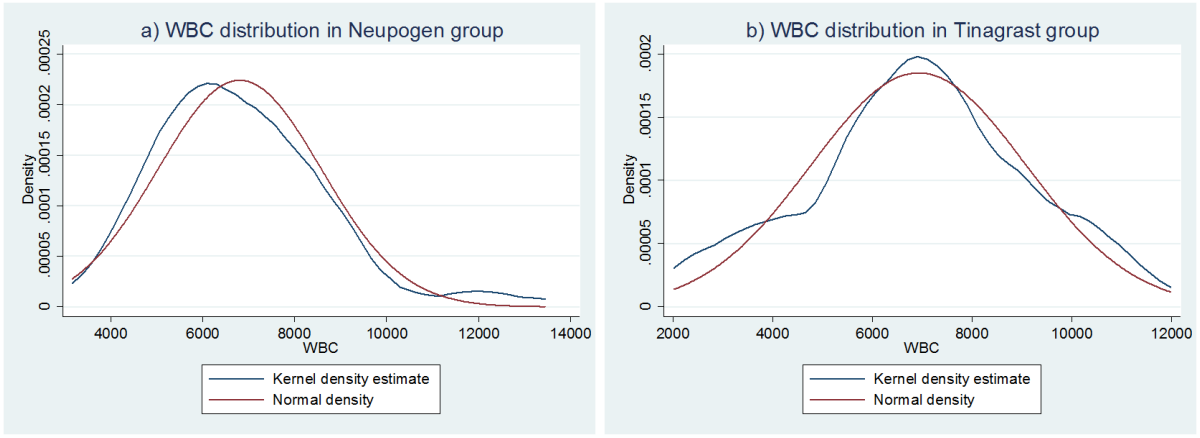

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Diagram 1: Evaluation of the variable normality of white blood cell count (per cubic millimeter) in the baseline phase based on treatment groups: a) Neupogen and b) Tinagrast

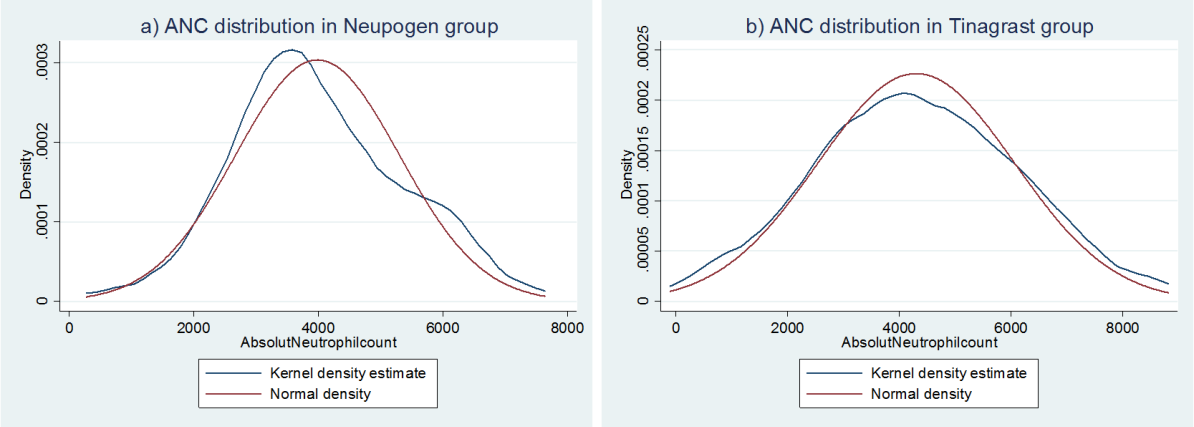

Diagram 2: Evaluation of the variable normality of Absolute Neutrophil Count (per cubic millimeter) in the baseline phase based on treatment groups: a) Neupogen and b) Tinagrast

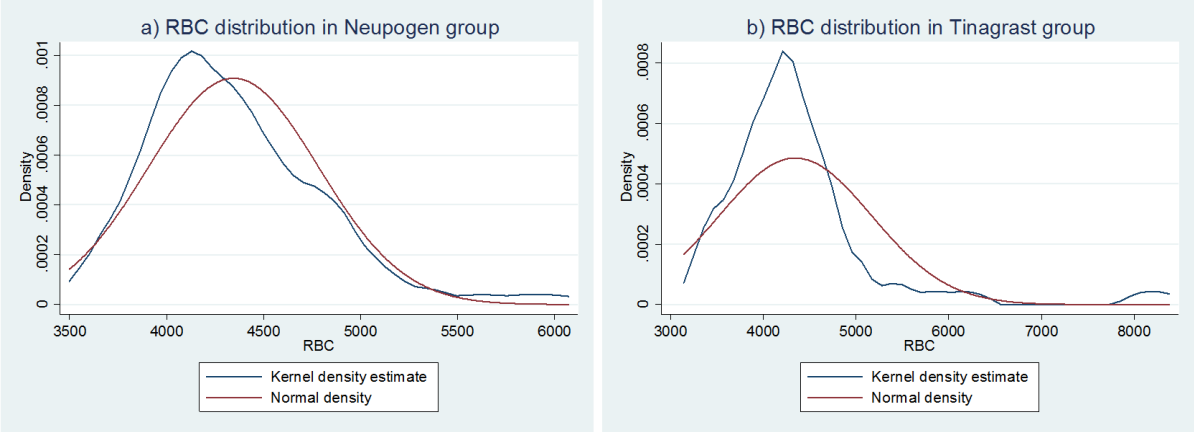

Diagram 3: Evaluation of the variable normality of red blood cell count (per cubic millimeter) in the baseline phase based on treatment groups: a) Neupogen and b) Tinagrast

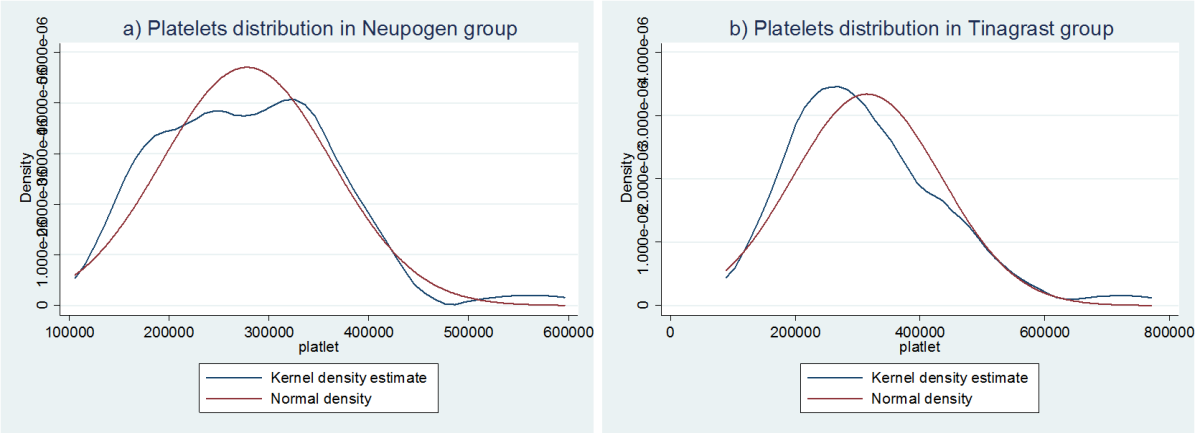

Diagram 1: Evaluation of the variable normality of platelets Count (per cubic millimeter) in the baseline phase based on treatment groups: a) Neupogen and b) Tinagrast

Interpretation of Figures 1 to 4:

As can be seen from the above four diagrams, white blood cell, neutrophil counts, and platelet count as three variables has a normal distribution (Kolmogorov-Smirnov normality test was not significant). However, red blood cell count, particularly in the Tinagrast group, was not normal. Therefore, as already seen in table 1, to make a comparison of the average or the distribution of red blood cells in the two groups, t-test was not used; an equivalent nonparametric test i.e. the Mann-Whitney was used instead.

c) The distribution of study outcomes in both groups during treatment cycles:

According to the executive protocol of the study, two treatment groups with Neupogen and Tinagrast were subject to clinical and paraclinical assessment and screening on day 1. All patients in the two treatment groups received a total of four injections in a one-week period. They were also subject to clinical and paraclinical assessment and screening on days 9, 22, 43 and 64 that, respectively, progressed along the first, second, third, and fourth treatment cycles.

Firstly, in Diagrams 5 to 8, distribution of white blood cell, the absolute neutrophil count, red blood cells, and platelets, as hematological parameters, were evaluated during the five previously-mentioned period, on a daily basis. To analyze the findings statistically, in other words, to assessing changes in average blood indexes, Repeated Measures ANOVA was used.

Not only is this his statistical method used to evaluate the treatments, but it also add the changes or repetitions occurred during the study.

In other words, in this method, it is possible to compare two primitive variables means (mean difference of each blood index in each group between each period and the baseline period and also the comparison between the two treatment groups at any period). This was done taking advantage of Post-hoc test (Scheffé's method) and taking the increase in Type I error due to multiple comparisons into control.

It should be noted that for every Diagram (5 to 8) a table describing the distribution of each blood index in cycles between the two treatment groups is allocated. Each table includes detailed report of the mean and standard error of the mean each period (a total of 10 mean and standard error of the for 10 time points that are the results of five periods in the two treatment groups). In addition, each table contains a report of the significance of the difference between the means in a binary fashion (a total of 13 differences between 13 p-values corresponding to these binary comparisons).

Since in the two treatment groups, four one-week cycles of treatment was used and clinical and paraclinical assessment and screening of blood index were done at the end of each cycle, it is advisable to examine the changes of these markers during these four period based on the two groups. Regarding the most prominent outcome of the present study (white blood cell count and absolute neutrophil count), the overall change in the indices is evaluated in the 5 periods. This is due to the fact that the evaluation done on the first day was added to these assessments.

To achieve the objective referred to in the previous paragraph, the best tool is a description of the error bar graph in which changes are shown. Diagram 5 and Table 2 show WBC changes, Diagram 6 and Table 3 illustrate ANC changes, Diagram 7 and Table 4 represent RBC changes, and finally Diagram 8 and Table 5 demonstrate platelet changes in the two treatment groups during the treatment period (5 mentioned periods).

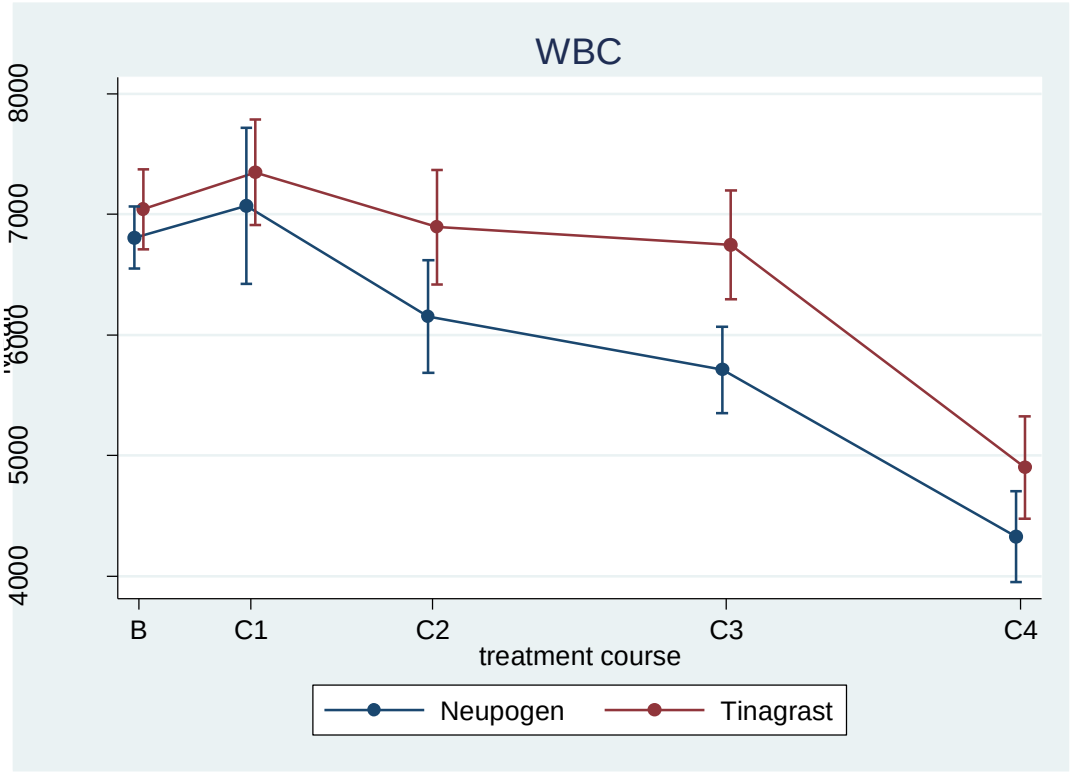

Diagram 5: Distribution of white blood cells based on treatment groups during treatment period patients:

B: Baseline period (day 1), C1: the end of the first treatment cycle (day 9), C2: the end of the second treatment cycle (day 22), C3: End the third treatment cycle (day 43), and C4: the end of the fourth treatment cycle (64 days)

Table 2) Distribution of the average white blood cell count at different times during the course of treatment based on the two treatment groups and the results of measuring the mean difference

p-value Between 2 groups &	P value Intergroup †	Treatment with Tinagrast	P value Intergroup †	Treatment with Neupogen	Time Period
۰.۷۰	–	۷۰۴۴±۳۳۲	–	۶۸۵۱±۲۵۵	Baseline (Day 1(
۰.۶۵	۰.۵۵	۷۳۴۸±۴۳۸	۰.۷۰	۶۹۸۷±۶۲۳	End of Cycle 1

					(Day 9)
۰.۲۳	۰.۷۷	۶۸۹۶±۴۷۵	۰.۱۳	۶۱۵۳±۴۶۸	End of Cycle 2 (Day 22)
۰.۱۰	۰.۵۷	۶۷۴۸±۴۴۹	۰.۰۱	۵۷۱۲±۳۵۸	End of Cycle 3 (Day 43)
۰.۳۶	۰.۰۰۱<	۴۹۰۱±۴۲۶	۰.۰۰۱<	۴۳۳۰±۳۷۶	End of Cycle 4 (Day 64)

†:p- value: Relating to the comparison between the blood cell indices at each specific time period (end of the four- cycle treatment) and the baseline period in each treatment groups

&: Relating to the comparison between the blood cell indices in two treatment groups at each of the five specific time periods (the baseline cycle and the end of four treatment cycle)

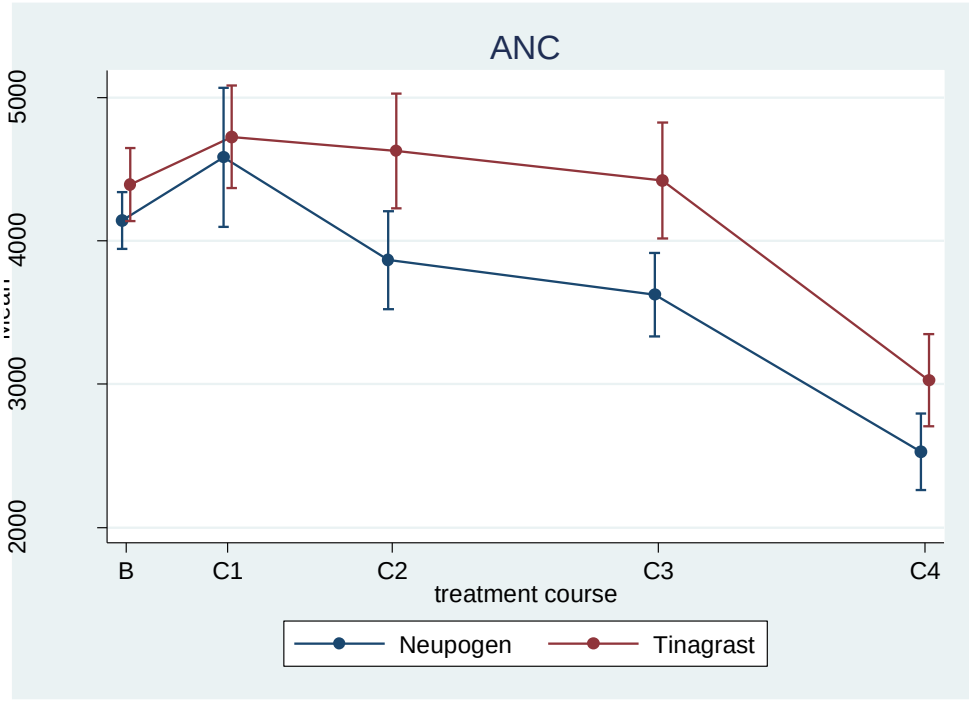

Diagram 6: Distribution of absolute neutrophil count based on treatment groups during treatment period patients:

B: Baseline period (day 1), C1: the end of the first treatment cycle (day 9), C2: the end of the second treatment cycle (day 22), C3: End the third treatment cycle (day 43), and C4: the end of the fourth treatment cycle (64 days)

Table 3) Distribution of the average absolute neutrophil count at different times during the course of treatment based on the two treatment groups and the results of measuring the mean difference

p-value Between 2 groups &	P value Intergroup †	Treatment with Tinagrast	P value Intergroup †	Treatment with Neupogen	Time Period
۰.۶۰	-	۴۳۹۳±۲۵۴	-	۴۱۷۸±۱۹۷	Baseline (Day 1(
۰.۷۷	۰.۴۲	۴۷۲۷±۳۵۸	۰.۳۵	۴۴۸۲±۴۷۱	End of Cycle 1 (Day 9)
۰.۱۲	۰.۵۷	۴۶۲۷±۴۰۲	۰.۳۶	۳۸۶۶±۳۴۲	End of Cycle 2 (Day 22)
۰.۱۰	۰.۹۵	۴۴۲۱±۴۰۳	۰.۱۱	۳۶۲۴±۲۹۰	End of Cycle 3 (Day 43)
۰.۳۱	۰.۰۰۱	۳۰۲۷±۳۲۱	۰.۰۰۱<	۲۵۲۸±۲۶۸	End of Cycle 4 (Day 64)

†:p- value: Relating to the comparison between the blood cell indices at each specific time period (end of the four- cycle treatment) and the baseline period in each treatment groups
&: Relating to the comparison between the blood cell indices in two treatment groups at each of the five specific time periods (the baseline cycle and the end of four treatment cycle)

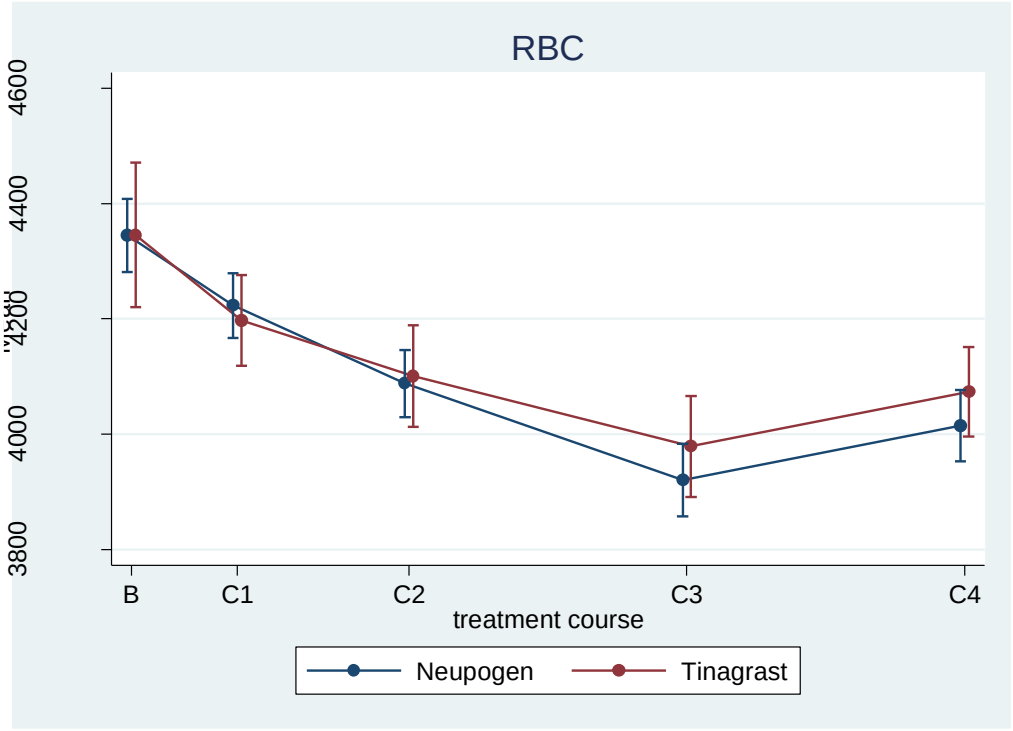

Diagram 7: Distribution of red blood cells based on treatment groups during treatment period patients:

B: Baseline period (day 1), C1: the end of the first treatment cycle (day 9), C2: the end of the second treatment cycle (day 22), C3: End the third treatment cycle (day 43), and C4: the end of the fourth treatment cycle (64 days)

Table 4) Distribution of the average red blood cell count (×۱۰۰)at different times during the course of treatment based on the two treatment groups and the results of measuring the mean difference

p-value Between 2 groups &	P value Intergroup †	Treatment with Tinagrast	P value Intergroup †	Treatment with Neupogen	Time Period
۰.۹۹	–	۴۳۴۶±۱۲۵	–	۴۳۴۳±۶۲	Baseline (Day

					1(
۰.۸۱	۰.۰۱	۴۱۹۷±۷۹	۰.۰۳	۴۲۱۹±۵۵	End of Cycle 1 (Day 9)
۰.۹۱	۰.۰۰۱<	۴۱۰۱±۸۸	۰.۰۰۱<	۴۰۸۸±۵۸	End of Cycle 2 (Day 22)
۰.۶۰	۰.۰۰۱<	۳۹۷۹±۸۸	۰.۰۰۱<	۳۹۲۰±۶۳	End of Cycle 3 (Day 43)
۰.۶۱	۰.۰۰۱<	۴۰۷۳±۷۸	۰.۰۰۱<	۴۰۱۴±۶۲	End of Cycle 4 (Day 64)

†:p- value: Relating to the comparison between the blood cell indices at each specific time period (end of the four- cycle treatment) and the baseline period in each treatment groups

&: Relating to the comparison between the blood cell indices in two treatment groups at each of the five specific time periods (the baseline cycle and the end of four treatment cycle)

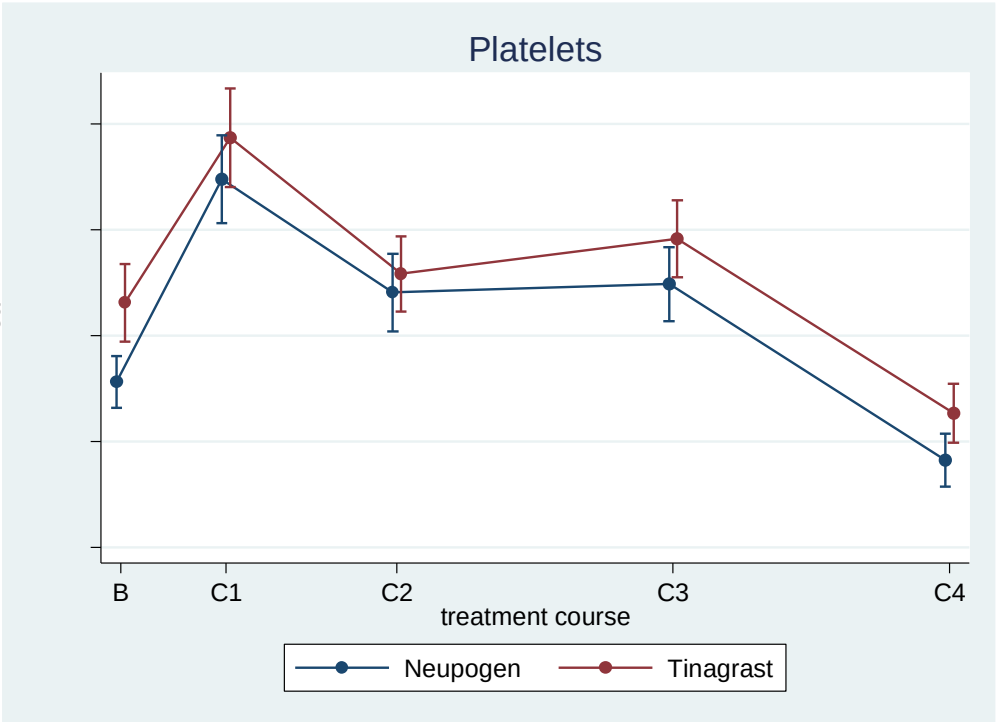

Diagram 8: Platelet distribution based on treatment groups during treatment period patients: B: Baseline period (day 1), C1: the end of the first treatment cycle (day 9), C2: the end of the second treatment cycle (day 22), C3: End the third treatment cycle (day 43), and C4: the end of the fourth treatment cycle (64 days)

Table 5) Distribution of the average platelet count (×۱۰۰۰) at different times during the course of treatment based on the two treatment groups and the results of measuring the mean difference

p-value Between 2 groups &	P value Intergroup †	Treatment with Tinagrast	P value Intergroup †	Treatment with Neupogen	Time Period
۰.۱۳	-	۳۱۵±۱۸	-	۲۷۸±۱۲	Baseline (Day 1(
۰.۴۳	۰.۰۰۱<	۳۹۳±۲۳	۰.۰۰۱<	۳۶۶±۲۱	End of Cycle 1 (Day 9)
۰.۷۲	۰.۴۵	۳۲۹±۱۸	۰.۰۲	۳۲۰±۱۸	End of Cycle 2 (Day 22)
۰.۴۰	۰.۱۰	۳۴۶±۱۸	۰.۰۱	۳۲۴±۱۸	End of Cycle 3 (Day 43)
۰.۳۸	۰.۰۰۴	۲۶۳±۱۴	۰.۰۲	۲۴۱±۱۲	End of Cycle 4 (Day 64)

†:p- value: Relating to the comparison between the blood cell indices at each specific time period (end of the four- cycle treatment) and the baseline period in each treatment groups
&: Relating to the comparison between the blood cell indices in two treatment groups at each of the five specific time periods (the baseline cycle and the end of four treatment cycle)

d) The distribution of study outcomes and adverse effects in both groups at the end of cycles 1 to 4:

Classified distribution of the study outcomes (different levels and different levels of neutrophils, white blood cells) is assessed; the corresponding effects are examined based on four separate periods in accordance with the end of cycles 1 to 4.

Fortunately, in the first follow-up period, no case of unavailability or loss to follow-up in patients was seen in either treatment groups. Findings of all patients assigned to a random treatment (in Neupogen and Tinagrast, respectively, 49 and 43) were also analyzed. The findings are summarized in Table 6.

As can be seen in Table 6, all outcomes and adverse effects in the two treatment groups were not significantly different ($p > 0.05$), apart from vomiting and muscle pain for which the frequency of occurrence in Tinagrast group is significantly lower than that of the Neupogen group ($p < 0.05$).

In the second follow-up period or the end of the second treatment cycle, which almost matched the day 22 after the beginning of treatment, 3 patients` information could not be accessed in Neupogen group since they were not available. In other words, in the two treatment groups there were, respectively, 46 and 43 participants. According to principles and standards of data analysis in clinical trials in terms of loss to follow-up or data missing, various strategies including Intentio To Treat (ITT) or Per-Protocol (PP) may be used.

In ITT solution, for all participants who entered and assigned to two treatment groups, statistical analysis was performed .In other words, the denominator in calculating incidence or prevalence of outcomes in this solution is all participants assigned (regardless of what would happen in the future for these people, or whether these participants leave the trial due to loss to follow-up phenomenon).

However, in PP solution, contrary to previously-mentioned guidelines, criteria for the analysis of data is completion or finishing study (or achieving a specific follow-up period, for example, second or third follow-up) . Additionally, those who could not complete the study for any reason or experienced loss to follow-up are excluded form data analysis.

In Table 7, data analysis in the third follow-up is summarized based on PP solution (analysis of Neupogen treatment groups with 46 participants and Tinagrast treatment groups with 43 participants). Obviously, data analysis based on ITT solution will be dealt with later in this study for the second, third, and fourth follow-ups.

Table 6: Distribution of serious outcomes and adverse effects in the groups of patients under study (receiving Neupogen and Tinagrast) based on the different levels in the first follow-up period (day 9 after the beginning of treatment)

p-value	All patients in both groups (n=92)	Tinagrast Group (n=43)	Neupogen Group (n=49)	Grades	Outcome/ effect
	۹۱ (۹۸.۹)	۴۳ (۱۰۰.۰)	۴۸ (۹۸.۰)	Grade 0/normal(over 1500)	ANC (mm3)
	۱ (۱.۱)	۰ (۰.۰)	۱ (۲.۰)	Grade 1 (1500-1000)	
۰.۵۳†	۰ (۰.۰)	۰ (۰.۰)	(0.0) ۰	Grade 2 (500-1000)	
	(0.0) ۰	(0.0) ۰	(0.0) ۰	Grade 3 (200-500)	
	(0.0) ۰	(0.0) ۰	(0.0) ۰	Grade 4 (less than 200)	
	۹۰ (۹۷.۸)	۴۲ (۹۷.۷)	۴۸ (۹۸.۰)	Grade 0/normal(over 3000)	WBC) (mm3
	۲ (۲.۲)	۱ (۲.۳)	۱ (۲.۰)	Grade 1 (2000-3000)	
۰.۹۳†	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (1500-2000)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (1000-1500)	

	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (less than 1000)	
	۸۴ (۹۱.۳)	۳۹ (۹۰.۷)	۴۵ (۹۱.۸)	Grade 0 / normal (over 150)	PLT ×1000 mm^3
	۸ (۸.۷)	۴ (۹.۳)	۴ (۸.۲)	Grade 1 (75-150)	
$۰.۸۵	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (50-75)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (10-50)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (less than 10)	
	۴۱ (۴۴.۶)	۱۸ (۴۱.۹)	۲۳ (۴۶.۹)	Grade 0 / normal (over 12)	Hb Mg/dl
	۲۶ (۲۸.۳)	۱۴ (۳۲.۵)	۱۲ (۲۴.۵)	Grade 1 (11-12)	
$۰.۹۲	۱۳ (۱۴.۱)	۶ (۱۴.۰)	۷ (۱۴.۳)	Grade 2 (10-11)	
	۸ (۸.۷)	۳ (۷.۰)	۵ (۱۰.۲)	Grade 3 (9-10)	
	۴ (۴.۳)	۲ (۴.۶)	۲ (۴.۱)	Grade 4 (less than 9)	
	۹۲ (۱۰۰.۰)	۴۳ (۱۰۰.۰)	۴۹ (۱۰۰.۰)	Grade 0 / Normal (non)	Thrombocytopenia and bleeding
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 1 (mild, no blood required(	
–	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (moderate, no blood required(	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (severe, no blood required(	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (urgent blood required(	
۰.۹۳†	۹۰ (۹۷.۸)	۴۲ (۹۷.۷)	۴۸ (۹۸.۰)	Grade 0 / Normal (non)	Febrile Neutropenia

	۲ (۲.۲)	۱ (۲.۳)	۱ (۲.۰)	Grade 1 (39-38°c)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (39.1-40°c)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (higher than 40°c, less than 1 day)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (higher than 40°c, more than 1 day)	
	۹۲ (۱۰۰.۰)	۴۳ (۱۰۰.۰)	۴۹ (۱۰۰.۰)	Grade 0 / Normal (non)	Fever without Neutropenia
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 1 (39-38°c)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (39.1-40°c)	
–	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (higher than 40°c, less than 1 day)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (higher than 40°c, more than 1 day)	
	۶۰ (۶۵.۲)	۲۷ (۶۲.۸)	۳۳ (۶۷.۴)	Grade 0 / Normal (non)	Nausea
	۲۵ (۲۷.۲)	۱۳ (۳۰.۲)	۱۲ (۲۴.۵)	Grade 1 (normal oral feeding)	
$۰.۷۴	۶ (۶.۵)	۳ (۷.۰)	۳ (۶.۱)	Grade 2 (reduced oral intake)	
	۱ (۱.۱)	۰ (۰.۰)	۱ (۲.۰)	Grade 3 (no oral intake, serum required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (no oral intake, urgent need for serum)	
$۰.۰۳	۸۲ (۸۹.۱)	۴۱ (۹۵.۳)	۴۱ (۸۳.۷)	Grade 0 / Normal (non)	Vomiting

	۷ (۷.۶)	۰ (۰.۰)	۷ (۱۴.۳)	Grade 1 (1 per day)	
	۳ (۳.۳)	۲ (۴.۷)	۱ (۲.۰)	Grade 2 (2-5 times a day, no treatment required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (6 times and more, treatment required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (emergency infusion required)	
	۷۰ (۷۶.۱)	۳۶ (۸۳.۷)	۳۴ (۶۹.۴)	Grade 0 / Normal (non)	Diarrhea
	۹ (۹.۸)	۴ (۹.۳)	۵ (۱۰.۲)	Grade 1 (less than 4 times a day)	
	۱۱ (۱۲.۰)	۲ (۴.۷)	۹ (۱۸.۴)	Grade 2 (4-6 times a day)	
†۰.۱۸	۲ (۲.۱)	۱ (۲.۳)	۱ (۲.۰)	Grade 3 (7 times or more, infusion required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (emergency infusion required)	
	۵۱ (۵۵.۴)	۲۸ (۶۵.۱)	۲۳ (۴۶.۹)	Grade 0 / Normal (non)	Bone pain
	۲۶ (۲۸.۳)	۱۲ (۲۷.۹)	۱۴ (۲۸.۶)	Grade 1 (no tranquilizer required)	
	۱۲ (۱۳.۰)	۲ (۴.۷)	۱۰ (۲۰.۴)	Grade 2 (controllable with tranquilizer)	
$۰.۱۱	۳ (۳.۳)	۱ (۲.۳)	۲ (۴.۱)	Grade 3 (repeated pain ,controllable with tranquilizer)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (repeated pain , uncontrollable with tranquilizer)	

	٥٣ (٥٧.٦)	٣٠ (٦٩.٨)	٢٣ (٤٦.٩)	Grade 0 / Normal (non)	Muscular pain
	٢٧ (٢٩.٤)	١٢ (٢٧.٩)	١٥ (٣٠.٧)	Grade 1 (no tranquilizer required)	
	٨ (٨.٧)	٠ (٠.٠)	٨ (١٦.٣)	Grade 2 (controllable with tranquilizer)	
†٠.٠١	٤ (٤.٣)	١ (٢.٣)	٣ (٦.١)	Grade 3 (repeated pain ,controllable with tranquilizer)	
	٠ (٠.٠)	٠ (٠.٠)	٠ (٠.٠)	Grade 4 (repeated pain , uncontrollable with tranquilizer)	
	٨٧ (٩٤.٥)	٤٢ (٩٧.٧)	٤٥ (٩١.٩)	Grade 0 / Normal (non)	Injection site reactions (redness, Cutaneous reactions)
	٤ (٤.٤)	١ (٢.٣)	٣ (٦.١)	Grade 1 (2.5 to 5 cm)	
	١ (١.١)	٠ (٠.٠)	١ (٢.٠)	Grade 1 (5.1 to 10 cm)	
†٠.٦٢	٠ (٠.٠)	٠ (٠.٠)	٠ (٠.٠)	Grade 3 (over 10 cm)	
	٠ (٠.٠)	٠ (٠.٠)	٠ (٠.٠)	Grade 4 (necrosis / dermatitis with desquamation)	

$:Using Pearson's chi-square test :† ، Using Fisher's exact test

Table 7: Distribution of serious outcomes and adverse effects in the groups of patients under study (receiving Neupogen and Tinagrast) based on the different levels in the second follow-up period (day 22 after the beginning of treatment)

p-value	All patients in both groups	Tinagrast Group (n=43)	Neupogen Group (n=49)	Grades	Outcome/ effect

	(n=89)				
	۸۸ (۹۸.۹)	۴۳ (۱۰۰.۰)	۴۵ (۹۷.۸)	Grade 0/normal(over 1500)	ANC (mm3)
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 1 (1500-1000)	
۰.۵۲†	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (500-1000)	
	۱ (۱.۱)	۰ (۰.۰)	۱ (۲.۲)	Grade 3 (200-500)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (less than 200)	
	۸۴ (۹۴.۴)	۴۲ (۹۷.۷)	۴۲ (۹۱.۳)	Grade 0/normal(over 3000)	WBC) (mm3
	۴ (۴.۵)	۱ (۲.۳)	۳ (۶.۵)	Grade 1 (2000-3000)	
۰.۶۲†	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (1500-2000)	
	۱ (۱.۱)	۰ (۰.۰)	۱ (۲.۲)	Grade 3 (1000-1500)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (less than 1000)	
	۸۶ (۹۶.۶)	۴۲ (۹۷.۷)	۴۴ (۹۵.۶)	Grade 0 / normal (over 150)	PLT ×1000 mm3
	۳ (۳.۴)	۱ (۲.۳)	۲ (۴.۴)	Grade 1 (75-150)	
$۰.۶۰	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (50-75)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (10-50)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (less than 10)	

	۳۰ (۳۳.۷)	۱۶ (۳۷.۲)	۱۴ (۳۰.۴)	Grade 0 / normal (over 12)	Hb Mg/dl
	۲۴ (۲۷.۰)	۱۰ (۲۳.۳)	۱۴ (۳۰.۴)	Grade 1 (11-12)	
$۰.۵۰	۲۲ (۲۴.۷)	۹ (۲۰.۹)	۱۳ (۲۸.۳)	Grade 2 (10-11)	
	۱۰ (۱۱.۲)	۷ (۱۶.۳)	۳ (۶.۵)	Grade 3 (9-10)	
	۳ (۳.۴)	۱ (۲.۳)	۲ (۴.۴)	Grade 4 (less than 9)	
	۸۹ (۱۰۰.۰)	۴۳ (۱۰۰.۰)	۴۶ (۱۰۰.۰)	Grade 0 / Normal (non)	Thrombocytopenia and bleeding
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 1 (mild, no blood required(	
–	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (moderate, no blood required(	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (severe, no blood required(	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (urgent blood required)	
	۸۸ (۹۸.۹)	۴۲ (۹۷.۷)	۴۶ (۱۰۰.۰)	Grade 0 / Normal (non)	Febrile Neutropenia
	۱ (۱.۱)	۱ (۲.۳)	۰ (۰.۰)	Grade 1 (39-38°c)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (39.1-40°c)	
۰.۴۸†	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (higher than 40°c, less than 1 day)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (higher than 40°c, more than 1 day)	
–	۸۹ (۱۰۰.۰)	۴۳ (۱۰۰.۰)	۴۶ (۱۰۰.۰)	Grade 0 / Normal	Fever without

				(non)	Neutropenia
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 1 (39-38°c)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (39.1-40°c)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (higher than 40°c, less than 1 day)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (higher than 40°c, more than 1 day)	
	۵۶ (۶۲.۹)	۲۶ (۶۰.۵)	۳۰ (۶۵.۲)	Grade 0 / Normal (non)	Nausea
	۲۴ (۲۷.۰)	۱۵ (۳۴.۹)	۹ (۱۹.۶)	Grade 1 (normal oral feeding)	
†۰.۰۵	۸ (۹.۰)	۱ (۲.۳)	۷ (۱۵.۲)	Grade 2 (reduced oral intake)	
	۱ (۱.۱)	۱ (۲.۳)	۰ (۰.۰)	Grade 3 (no oral intake, serum required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (no oral intake, urgent need for serum)	
	۷۶ (۸۵.۴)	۳۸ (۸۸.۴)	۳۸ (۸۲.۶)	Grade 0 / Normal (non)	Vomiting
	۹ (۱۰.۱)	۵ (۱۱.۶)	۴ (۸.۷)	Grade 1 (1 per day)	
†۰.۱۷	۴ (۴.۵)	۰ (۰.۰)	۴ (۸.۷)	Grade 2 (2-5 times a day, no treatment required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (6 times and more, treatment required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (emergency infusion required)	

	۸۲ (۹۲.۱)	۴۲ (۹۷.۷)	۴۰ (۸۷.۰)	Grade 0 / Normal (non)	Diarrhea
	۵ (۵.۶)	۰ (۰.۰)	۵ (۱۰.۹)	Grade 1 (less than 4 times a day)	
	۲ (۲.۳)	۱ (۲.۳)	۱ (۲.۱)	Grade 2 (4-6 times a day)	
†۰.۰۶					
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (7 times or more, infusion required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (emergency infusion required)	
	۵۶ (۶۲.۹)	۲۹ (۶۷.۴)	۲۷ (۵۸.۷)	Grade 0 / Normal (non)	Bone pain
	۲۲ (۲۴.۷)	۱۰ (۲۳.۳)	۱۲ (۲۶.۱)	Grade 1 (no tranquilizer required)	
	۸ (۹.۰)	۳ (۷.۰)	۵ (۱۰.۹)	Grade 2 (controllable with tranquilizer)	
†۰.۸۵	۳ (۳.۴)	۱ (۲.۳)	۲ (۴.۳)	Grade 3 (repeated pain ,controllable with tranquilizer)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (uncontrollable with tranquilizer)	
	۶۰ (۶۷.۴)	۳۳ (۷۶.۷)	۲۷ (۵۸.۷)	Grade 0 / Normal (non)	Muscular pain
	۱۷ (۱۹.۱)	۷ (۱۶.۳)	۱۰ (۲۱.۷)	Grade 1 (no tranquilizer required)	
†۰.۲۹	۸ (۹.۰)	۲ (۴.۷)	۶ (۱۳.۰)	Grade 2 (controllable with tranquilizer)	
	۴ (۴.۵)	۱ (۲.۳)	۳ (۶.۵)	Grade 3 (repeated pain ,controllable with tranquilizer)	

	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (uncontrollable with tranquilizer)	
†۰.۳۶	۸۶ (۹۶.۶)	۴۲ (۹۷.۷)	۴۴ (۹۵.۷)	Grade 0 / Normal (non)	Injection site reactions (redness, Cutaneous reactions)
	۲ (۲.۳)	۰ (۰.۰)	۲ (۴.۳)	Grade 1 (2.5 to 5 cm)	
	۱ (۱.۱)	۱ (۲.۳)	۰ (۰.۰)	Grade 1 (5.1 to 10 cm)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (over 10 cm)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (necrosis / dermatitis with desquamation)	

$:Using Pearson's chi-square test :† ، Using Fisher's exact test

As can be seen in Table 7, no significant difference can be seen regarding the important outcomes including the normal range for the white blood cell count, normal neutrophils count range, and normal platelet count between the two groups (p> 0.05) despite the fact that the frequency of normal range in Tinagrast group was greater than that of Neupogen group. No significant difference was seen regarding the adverse effects (p> 0.05), except for the nausea (grade 2 and 3) in Neupogen group that was about 3 times more frequent than that of Tinagrast groups; this difference was statistically significant based on Fisher's exact test (p = 0.05).

In the third follow-up or at the end of the third treatment cycle (day 43 after the beginning of the treatment), two cases were lost to follow-up and were added to three previous cases. In other words, there was a total loss of five cases; therefore, the number of participants in the two treatment groups fell to 44 and 43, respectively (a total of 88).

Table 8 summarizes the different levels of outcomes and adverse effect. Almost 95 percent of people in the two treatment groups in terms of white blood cell count , neutrophils counts and platelet count are in the normal group. However, the frequency of normal or desirable count in the blood cells in the two treatment groups showed no statistically significant difference (p> 0.05). Regarding the frequency of adverse effects, some effects were not observed in any of the two groups (such as fever with or without neutropenia,

thrombocytopenia, and bleeding). Taking into account other adverse effect, no statistically significant difference was seen (p> 0.05).

In the last follow-up or at the end of the fourth cycle (approximately 64 days after the beginning of treatment), 1 other participant in the Neupogen group, merely tor measure blood cells (white blood cells, neutrophils and platelet counts) and 2 participants to assess adverse effects were excluded from the study in addition to the previous ones. In other words, the number of cases of missing or loss to follow-up in this group therapy increased to 6 (and 7). Therefore, in this phase, the number of participants in the two treatment groups of fell to 43 (total n = 86/85). Due to this reduction, the findings of the comparisng outcomes and adverse effects in the two treatment groups at this point is summarized in Table 9 based on the PP solution.

In this period, more than 70 percent of the two treatment groups were in normal situation, regarding the main outcomes of blood cells and no statistically significant difference was seen between the two groups (p> 0.05). Except for the frequency of vomiting (as a side effect) that was significantly lower in Tinagrast than in Neupogen group, the rest of the side effects were not statistically significant different (p> 0.05).

Table 8: Distribution of serious outcomes and adverse effects in the groups of patients under study (receiving Neupogen and Tinagrast) based on the different levels in the third follow-up period (day 43 after the beginning of treatment)

p-value	All patients in both groups (n=87)	Tinagrast Group (n=43)	Neupogen Group (n=44)	Grades	Outcome/ effect
۱.۰۰†	۸۵ (۹۷.۶)	۴۲ (۹۷.۷)	۴۳ (۹۷.۷)	Grade 0/normal(over 1500)	ANC (mm^{3})
	۱ (۱.۲)	۰ (۰.۰)	۱ (۲.۳)	Grade 1 (1500-	

				1000)	
	۱ (۱.۲)	۱ (۲.۳)	۰ (۰.۰)	Grade 2 (500-1000)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (200-500)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (less than 200)	
	۸۴ (۹۶.۶)	۴۲ (۹۷.۷)	۴۲ (۹۵.۵)	Grade 0/normal(over 3000)	WBC (mm^3)
	۳ (۳.۴)	۱ (۲.۳)	۲ (۴.۵)	Grade 1 (2000-3000)	
۰.۵۱†	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (1500-2000)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (1000-1500)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (less than 1000)	
	۸۶ (۹۸.۹)	۴۲ (۹۷.۷)	۴۴ (۱۰۰.۰)	Grade 0 / normal (over 150)	PLT $\times 1000\ mm^3$
	۱ (۱.۱)	۱ (۲.۳)	۰ (۰.۰)	Grade 1 (75-150)	
†۰.۴۹	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (50-75)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (10-50)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (less than 10)	
	۲۱ (۲۴.۱)	۱۰ (۲۳.۳)	۱۱ (۲۵.۰)	Grade 0 / normal (over 12)	Hb Mg/dl
	۲۶ (۲۹.۹)	۱۲ (۲۷.۹)	۱۴ (۳۱.۸)	Grade 1 (11-12)	
$۰.۷۲	۲۱ (۲۴.۱)	۹ (۲۰.۹)	۱۲ (۲۷.۳)	Grade 2 (10-11)	
	۱۷ (۱۹.۶)	۱۱ (۲۵.۶)	۶ (۱۳.۶)	Grade 3 (9-10)	
	۲ (۲.۳)	۱ (۲.۳)	۱ (۲.۳)	Grade 4 (less than 9)	
–	۸۵ (۱۰۰.۰)	۴۳ (۱۰۰.۰)	۴۲ (۱۰۰.۰)	Grade 0 / Normal (non)	Thrombocytopenia

	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 1 (mild, no blood required)	and bleeding
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (moderate, no blood required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (severe, no blood required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (urgent blood injection required)	
	۸۵ (۱۰۰.۰)	۴۳ (۱۰۰.۰)	۴۲ (۱۰۰.۰)	Grade 0 / Normal (non)	Febrile Neutropenia
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 1 (39-38°c)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (39.1-40°c)	
–	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (higher than 40°c, less than 1 day)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (higher than 40°c, more than 1 day)	
	۸۷ (۱۰۰.۰)	۴۳ (۱۰۰.۰)	۴۴ (۱۰۰.۰)	Grade 0 / Normal (non)	Fever without Neutropenia
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 1 (39-38°c)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (39.1-40°c)	
–	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (higher than 40°c, less than 1 day)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (higher than 40°c, more than 1 day)	
†۰.۱۸	۵۷ (۶۵.۵)	۲۶ (۶۰.۵)	۳۱ (۷۰.۴)	Grade 0 / Normal (non)	Nausea
	۲۳ (۲۶.۴)	۱۵ (۳۴.۹)	۸ (۱۸.۲)	Grade 1 (normal oral	

				feeding)	
	۵ (۵.۸)	۱ (۲.۳)	۴ (۹.۱)	Grade 2 (reduced oral intake)	
	۲ (۲.۳)	۱ (۲.۳)	۱ (۲.۳)	Grade 3 (no oral intake, serum required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (no oral intake, urgent need for serum)	
	۷۷ (۸۸.۵)	۳۹ (۹۰.۷)	۳۸ (۸۶.۴)	Grade 0 / Normal (non)	Vomiting
	۸ (۹.۲)	۴ (۹.۳)	۴ (۹.۱)	Grade 1 (1 per day)	
†۰.۶۳	۲ (۲.۳)	۰ (۰.۰)	۲ (۴.۵)	Grade 2 (2-5 times a day, no treatment required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (6 times and more, treatment required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (emergency infusion required)	
	۷۷ (۸۸.۵)	۳۸ (۸۸.۴)	۳۹ (۸۸.۶)	Grade 0 / Normal (non)	Diarrhea
	۸ (۹.۲)	۳ (۷.۰)	۵ (۱۱.۴)	Grade 1 (less than 4 times a day)	
†۰.۴۹	۲ (۲.۳)	۲ (۴.۶)	۰ (۰.۰)	Grade 2 (4-6 times a day)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (7 times or more, infusion required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (emergency infusion required)	
	۵۴ (۶۲.۱)	۳۰ (۶۹.۸)	۲۴ (۵۴.۶)	Grade 0 / Normal (non)	Bone pain
†۰.۲۴	۲۵ (۲۸.۷)	۱۰ (۲۳.۳)	۱۵ (۳۴.۱)	Grade 1 (no tranquilizer required)	

	۷ (۸.۱)	۲ (۴.۷)	۵ (۱۱.۴)	Grade 2 (controllable with tranquilizer)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (repeated pain ,controllable with tranquilizer)	
	۱ (۱.۱)	۱ (۲.۳)	۰ (۰.۰)	Grade 4 (repeated pain , uncontrollable with tranquilizer)	
	۴۷ (۵۴.۰)	۲۸ (۶۵.۱)	۱۹ (۴۳.۲)	Grade 0 / Normal (non)	Muscular pain
	۳۲ (۳۶.۸)	۱۲ (۲۷.۹)	۲۰ (۴۵.۵)	Grade 1 (no tranquilizer required)	
	۶ (۶.۹)	۲ (۴.۷)	۴ (۹.۰)	Grade 2 (controllable with tranquilizer)	
†۰.۱۷	۲ (۲.۳)	۱ (۲.۳)	۱ (۲.۳)	Grade 3 (repeated pain ,controllable with tranquilizer)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (repeated pain , uncontrollable with tranquilizer)	
	۸۲ (۹۴.۲)	۴۱ (۹۵.۴)	۴۱ (۹۳.۲)	Grade 0 / Normal (non)	Injection site reactions (redness, Cutaneous reactions(
	۴ (۴.۶)	۲ (۴.۷)	۲ (۴.۶)	Grade 1 (2.5 to 5 cm)	
	۱ (۱.۲)	۰ (۰.۰)	۱ (۲.۳)	Grade 1 (5.1 to 10 cm)	
†۰.۴۴	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (over 10 cm)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (necrosis / dermatitis with desquamation)	

$: Using Pearson's chi-square test ،†: Using Fisher's exact test

Table 9: Distribution of serious outcomes and adverse effects in the groups of patients under study (receiving Neupogen and Tinagrast) based on the different levels in the fourth follow-up period (day 64 after the beginning of treatment)

p-value	All patients in both groups (n=86)	Tinagrast Group (n=43)	Neupogen Group (n=43)	Grades	Outcome/ effect
	۶۵ (۷۵.۶)	۳۲ (۷۴.۴)	۳۳ (۷۶.۷)		ANC (mm3)
	۷ (۸.۱)	۴ (۹.۳)	۳ (۷.۰)	Grade 0/normal(over 1500)	
۰.۷۹†	۸ (۹.۳)	۵ (۱۱.۶)	۳ (۷.۰)	Grade 1 (1500-1000)	
	۶ (۷.۰)	۲ (۴.۷)	۴ (۹.۳)	Grade 2 (500-1000)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (200-500)	
	۶۵ (۷۵.۶)	۳۲ (۷۴.۴)	۳۳ (۷۶.۷)	Grade 4 (less than 200)	WBC) (mm3
	۱۳ (۱۵.۱)	۷ (۱۶.۳)	۶ (۱۳.۹)		
۱.۰۰†	۵ (۵.۸)	۳ (۷.۰)	۲ (۴.۷)	Grade 0/normal(over 3000)	
	۳ (۳.۵)	۱ (۲.۳)	۲ (۴.۷)	Grade 1 (2000-3000)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (1500-2000)	
	۷۹ (۹۱.۹)	۳۸ (۸۸.۴)	۴۱ (۹۵.۳)	Grade 3 (1000-1500)	
	۶ (۷.۰)	۴ (۹.۳)	۲ (۴.۷)	Grade 4 (less than 1000)	PLT ×1000 mm3
†۰.۴۳	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)		
	۱ (۱.۱)	۱ (۲.۳)	۰ (۰.۰)	Grade 0 / normal (over 150)	

	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 1 (75-150)	
	۱۹ (۲۲.۴)	۱۰ (۲۳.۳)	۹ (۲۱.۴)	Grade 2 (50-75)	Hb Mg/dl
	۱۸ (۲۱.۲)	۸ (۱۸.۶)	۱۰ (۲۳.۸)	Grade 2 (10-50)	
$۰.۹۱	۲۷ (۳۱.۷)	۱۳ (۳۰.۲)	۱۴ (۳۳.۳)	Grade 4 (less than 10)	
	۱۵ (۱۷.۶)	۸ (۱۸.۶)	۷ (۱۶.۷)		
	۶ (۷.۱)	۴ (۹.۳)	۲ (۴.۸)	Grade 0 / normal (over 12)	
	۸۷ (۱۰۰.۰)	۴۳ (۱۰۰.۰)	۴۴ (۱۰۰.۰)	Grade 1 (11-12)	Thrombocytopenia and bleeding
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (10-11)	
_	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (9-10)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (less than 9)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 0 / Normal (non)	
	۸۷ (۱۰۰.۰)	۴۳ (۱۰۰.۰)	۴۴ (۱۰۰.۰)	Grade 1 (mild, no blood required)	Febrile Neutropenia
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 2 (moderate, no blood required)	
_	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (severe, no blood required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (urgent blood required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 0 / Normal (non)	
	۸۴ (۹۸.۸)	۴۳ (۱۰۰.۰)	۴۱ (۹۷.۶)	Grade 1 (39-38°c)	Fever without Neutropenia
۰.۴۹†	۱ (۱.۲)	۰ (۰.۰)	۱ (۲.۳)	Grade 2 (39.1-40°c)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (higher than 40°c, less than 1 day)	

	٠ (٠.٠)	٠ (٠.٠)	٠ (٠.٠)	Grade 4 (higher than 40°c, more than 1 day)	
	٠ (٠.٠)	٠ (٠.٠)	٠ (٠.٠)	Grade 0 / Normal (non)	

	۵۹ (۶۹.۴)	۲۹ (۶۷.۴)	۳۰ (۷۱.۴)	Grade 0 / Normal (non)	Nausea
	۲۰ (۲۳.۵)	۱۱ (۲۵.۶)	۹ (۲۱.۴)	Grade 1 (normal oral feeding)	
†۰.۹۶	۴ (۴.۷)	۲ (۴.۷)	۲ (۴.۸)	Grade 2 (reduced oral intake)	
	۲ (۲.۴)	۱ (۲.۳)	۱ (۲.۴)	Grade 3 (no oral intake, serum required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (no oral intake, urgent need for serum)	
	۷۴ (۸۷.۱)	۴۱ (۹۵.۴)	۳۳ (۷۸.۶)	Grade 0 / Normal (non)	Vomiting
	۸ (۹.۴)	۱ (۲.۳)	۷ (۱۶.۶)	Grade 1 (1 per day)	
	۳ (۳.۵)	۱ (۲.۳)	۲ (۴.۸)	Grade 2 (2-5 times a day, no treatment required)	
†۰.۰۳	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (6 times and more, treatment required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (emergency infusion required)	
	۷۳ (۸۵.۹)	۳۶ (۸۳.۷)	۳۷ (۸۸.۱)	Grade 0 / Normal (non)	Diarrhea
	۱۱ (۱۲.۹)	۶ (۱۴.۰)	۵ (۱۱.۹)	Grade 1 (less than 4 times a day)	
†۱.۰۰	۱ (۱.۲)	۱ (۲.۳)	۰ (۰.۰)	Grade 2 (4-6 times a day)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 3 (7 times or more, infusion required)	
	۰ (۰.۰)	۰ (۰.۰)	۰ (۰.۰)	Grade 4 (emergency infusion required)	
†۰.۱۹	۵۱ (۶۰.۰)	۲۶ (۶۰.۵)	۲۵ (۵۹.۵)	Grade 0 / Normal (non)	Bone pain

	٢٠ (٢٣.٥)	٧ (١٦.٣)	١٣ (٣١.٠)	Grade 1 (no tranquilizer required)	
	٨ (٩.٤)	٥ (١١.٦)	٣ (٧.١)	Grade 2 (controllable with tranquilizer)	
	٦ (٧.١)	٥ (١١.٦)	١ (٢.٤)	Grade 3 (repeated pain ,controllable with tranquilizer)	
	٠ (٠.٠)	٠ (٠.٠)	٠ (٠.٠)	Grade 4 (repeated pain , uncontrollable with tranquilizer)	
	٥٣ (٦٢.٤)	٣٠ (٦٩.٨)	٢٣ (٥٤.٨)	Grade 0 / Normal (non)	Muscular pain
	٢٢ (٢٥.٩)	٨ (١٨.٦)	١٤ (٣٣.٣)	Grade 1 (no tranquilizer required)	
	٧ (٨.٢)	٢ (٤.٦)	٥ (١١.٩)	Grade 2 (controllable with tranquilizer)	
†٠.٠٨	٣ (٣.٥)	٣ (٧.٠)	٠ (٠.٠)	Grade 3 (repeated pain ,controllable with tranquilizer)	
	٠ (٠.٠)	٠ (٠.٠)	٠ (٠.٠)	Grade 4 (repeated pain , uncontrollable with tranquilizer)	
	٨٤ (٩٨.٨)	٤٢ (٩٧.٧)	٤٢ (١٠٠.٠)	Grade 0 / Normal (non)	Injection site reactions (redness, Cutaneous reactions)
	١ (١.٢)	١ (٢.٣)	٠ (٠.٠)	Grade 1 (2.5 to 5 cm)	
	٠ (٠.٠)	٠ (٠.٠)	٠ (٠.٠)	Grade 1 (5.1 to 10 cm)	
†٠.٥١	٠ (٠.٠)	٠ (٠.٠)	٠ (٠.٠)	Grade 3 (over 10 cm)	
	٠ (٠.٠)	٠ (٠.٠)	٠ (٠.٠)	Grade 4 (necrosis / dermatitis with desquamation)	

$Using Pearson's chi-square test ،†: Using Fisher's exact test

e) The distribution of main outcomes of the study in the two treatment groups based on treatment cycles 1 to 4 using ITT solution

According to what was previously stated about two main strategies for data analysis in clinical trials, during the execution of the study, a study protocol deviation may occur for any reason (this deviation can be loss to follow-up, switch between the two treatment groups due to occurrence of a side effect, etc.). In the following section, the data are analyzed according to ITT solution:
To analyze the data more precisely and to make a more comprehensive assessment of the impact of the loss to follow-up on the findings of this study , different scenarios contributed to the analysis of the data

In scenario 1, the denominator or the total population in Neupogen group were all participants being studied in baseline phase (and also the first follow-up for no missing had occurred before), and treatment success was defined by dividing the number of participants with normal blood cell by all participants. In other words, the denominator was regarded as index of treatment success in Neupogen and Tinagrast treatment groups (49 and 43 participants, respectively).

In scenario 2, Last Observation Carried Forward (LOCF) method was used to approach missing as a problem. In this method, the condition of the missed participants is replaced with the last condition of the outcome (the previous follow-up). For instance, if a participant is not available in the second follow-up and the main outcome of this person is missing while the white blood cell count in this person is of grade 1 (white blood cell count 3000-2000 per cubic millimeter of blood), this outcome is replaced in missing period.

In scenario 3, Worst Outcome Imputation (WOI) was used to approach missing as a problem. In this method, contrary to the previous one, the missed data is not replaces with the data of

the last situation, but the worst or the most undesirable situation of outcome is replaced instead.

Table 10: Comparing the ratio of treatment success outcomes based on the absolute number of neutrophils at different study periods using PP solution

Treatment Group	Period B total population /normal participants (risk)	Period C1 total population /normal participants (risk)	Period C2 total population /normal participants (risk)	Period C3 total population /normal participants (risk)	Period C4 total population /normal participants (risk)
Neupogen	۴۹ / ۴۹ (۱.۰)	۴۸ / ۴۹ (۰.۹۸)	۴۵ / ۴۶ (۰.۹۸)	۴۳ / ۴۴ (۰.۹۸)	۳۳ / ۴۳ (۰.۷۷)
Tinagrast	۴۲ / ۴۳ (۰.۹۸)	۴۳ / ۴۳ (۱.۰)	۴۳ / ۴۳ (۱.۰)	۴۲ / ۴۳ (۰.۹۸)	۳۲ / ۴۳ (۰.۷۴)
Risk Ratio	۱.۰۲ (۰.۹۸-۱.۰۷)	۰.۹۸ (۰.۹۴-۱.۰۲)	۰.۹۸ (۰.۹۴-۱.۰۲)	۱.۰۰ (۰.۹۴-۱.۰۷)	۱.۰۳ (۰.۸۱-۱.۳۱)
Risk Difference	۰.۰۲ (-۰.۰۲_۰.۰۷)	-۰.۰۲ (-۰.۰۶_۰.۰۲)	-۰.۰۲ (-۰.۰۶_۰.۰۲)	۰.۰۰ (-۰.۰۷_۰.۰۷)	۰.۰۲ (-۰.۱۵_۰.۲۱)

Table 11: Comparing the ratio of treatment success outcomes based on the absolute number of neutrophils at different study periods using ITT solution (Scenario 1)

Treatment Group	Period B total population	Period C1 total population	Period C2 total population	Period C3 total population	Period C4 total population

	/normal participants (risk)	/normal participants (risk)	/normal participants (risk)	/normal participants (risk)	/normal participants (risk)
Neupogen	۴۹ / ۴۹ (۱.۰)	۴۸ / ۴۹ (۰.۹۸)	۴۵ / ۴۹ (۰.۹۲)	۴۳ / ۴۹ (۰.۸۸)	۳۳ / ۴۹ (۰.۶۷)
Tinagrast	۴۲ / ۴۳ (۰.۹۸)	۴۳ / ۴۳ (۱.۰)	۴۳ / ۴۳ (۱.۰)	۴۲ / ۴۳ (۰.۹۸)	۳۲ / ۴۳ (۰.۷۴)
Risk Ratio	۱.۰۲ (۰.۹۸–۱.۰۷)	۰.۹۸ (۰.۹۴–۱.۰۲)	۰.۹۲ (۰.۸۵–۱.۰۰)	۰.۹۰ (۰.۸۰–۱.۰۱)	۰.۹۱ (۰.۷۰–۱.۱۸)
Risk Difference	۰.۰۲ (–۰.۰۲_۰.۰۷)	–۰.۰۲ (–۰.۰۶_۰.۰۲)	–۰.۰۸ (–۰.۱۶_–۰.۰۱)	–۰.۱۰ (–۰.۲۰_۰.۰۰۳)	–۰.۰۷ (–۰.۲۶_۰.۱۱)

Table 12: Comparing the ratio of treatment success outcomes based on the absolute number of neutrophils at different study periods using ITT solution (Scenario 2)

Treatment Group	Period B total population /normal participants (risk)	Period C1 total population /normal participants (risk)	Period C2 total population /normal participants (risk)	Period C3 total population /normal participants (risk)	Period C4 total population /normal participants (risk)
Neupogen	۴۹ / ۴۹ (۱.۰)	۴۸ / ۴۹ (۰.۹۸)	۴۸ / ۴۹ (۰.۹۸)	۴۸ / ۴۹ (۰.۹۸)	۴۸ / ۴۹ (۰.۹۸)
Tinagrast	۴۲ / ۴۳ (۰.۹۸)	۴۳ / ۴۳ (۱.۰)	۴۳ / ۴۳ (۱.۰)	۴۲ / ۴۳ (۰.۹۸)	۳۲ / ۴۳ (۰.۷۴)
Risk Ratio	۱.۰۲ (۰.۹۸–۱.۰۷)	۰.۹۸ (۰.۹۴–۱.۰۲)	۰.۹۸ (۰.۹۴–۱.۰۲)	۱.۰۰ (۰.۹۴–۱.۰۷)	۱.۳۲ (۱.۱۰–۱.۵۸)
Risk Difference	۰.۰۲	–۰.۰۲	–۰.۰۲	۰.۰۰۳	۰.۲۴

	(-۰.۰۲_۰.۰۷)	(-۰.۰۶_۰.۰۲)	(-۰.۰۶_۰.۰۲)	(-۰.۰۶_۰.۰۶)	(۰.۱۰_۰.۳۷)

Table 13: Comparing the ratio of treatment success outcomes based on the absolute number of neutrophils at different study periods using ITT solution (Scenario 3)

Treatment Group	Period B total population /normal participants (risk)	Period C1 total population /normal participants (risk)	Period C2 total population /normal participants (risk)	Period C3 total population /normal participants (risk)	Period C4 total population /normal participants (risk)
Neupogen	۴۹ / ۴۹ (۱.۰)	۴۸ / ۴۹ (۰.۹۸)	۴۸ / ۴۹ (۰.۹۸)	۴۸ / ۴۹ (۰.۹۸)	۴۸ / ۴۹ (۰.۹۸)
Tinagrast	۴۲ / ۴۳ (۰.۹۸)	۴۳ / ۴۳ (۱.۰)	۴۳ / ۴۳ (۱.۰)	۴۲ / ۴۳ (۰.۹۸)	۳۲ / ۴۳ (۰.۷۴)
Risk Ratio	۱.۰۲ (۰.۹۸-۱.۰۷)	۰.۹۸ (۰.۹۴-۱.۰۲)	۰.۹۸ (۰.۹۴-۱.۰۲)	۱.۰۰ (۰.۹۴-۱.۰۷)	۱.۳۲ (۱.۱۰-۱.۵۸)
Risk Difference	۰.۰۲ (-۰.۰۲_۰.۰۷)	-۰.۰۲ (-۰.۰۶_۰.۰۲)	-۰.۰۲ (-۰.۰۶_۰.۰۲)	۰.۰۰۳ (-۰.۰۶_۰.۰۶)	۰.۲۴ (۰.۱۰_۰.۳۷)

Scenario 2 and 3 in ITT led to the same findings.

However, in most periods and in most solutions, no significant differences was seen between the two treatment groups ;it can be concluded that the results of treatment using these two drugs were similar.

Discussion and conclusion

The present randomized clinical trial was designed and executed in pursuance of making a comparative evaluation of the effects of Neupogen and Tinagrast on Febrile Neutropenia following common chemotherapy in patients with breast cancer. The findings of this trial showed that in the first, second, and third follow-up periods, in the two treatment groups, more than 95% of participants reached their normal neutrophil level (index: absolute neutrophil count above 1,500 per cubic millimeter of blood). It was only in the fourth follow-up that the neutrophil level in these two groups was lower than that of previous periods. However, no statistically significant difference was seen (in Neupogen and Tinagrast treatment groups, respectively, 76.7 and 74.4 percent of participants had normal neutrophil level) ($p> 0.05$).

Although due to the prospective nature of this clinical trial, like many other clinical trials, the phenomenon of the loss of some subjects or loss to follow-up, the probability of biased selection in these studies will increase, this phenomenon is influenced by two factors i.e. the rate of lost participants as well as their relationship with the determinant factors to therapeutic response. Fortunately, regarding the first factor or proportion of lost people, 6 cases in Neupogen treatment until the end of the fourth track is not significant (12.4% compared to the first sample in Neupogen group). Furthermore, the distribution of background variables of age, staging and distribution, as well as other important variables such as white blood cell distribution or absolute neutrophil count (as the second issue) in the baseline phase, in both groups (lost and without this phenomenon) (people whose information was available until the last period) were not statistically significant. Thus, it can be concluded that the phenomenon of loss to follow-up has no significant effect on the results of this trial.

In the present clinical trial, a variety of approaches and solutions (as recommended in clinical trial references) has been implemented to analyze the results, i.e. Per Protocol (PP) and Intention To Treat (ITT). To go beyond these two mentioned approaches, the replacement of Missing data in lost people was done using two solutions: replacement with the previous observation (or previous follow-up) or replacement with the worst or the most undesirable previous status. After reviewing the findings from analyses data and comparing the main result of the study (normal neutrophil count) in the last follow-up or at the end of cycle 4, it was found that PP and ITT methods (scenario 1) lead to the same results. In these methods, no significant difference was seen between the two treatment group with regard to the main outcome as an index in no follow-up. In addition, since in most references, ITT is referred to as the most appropriate and valid method to obtain results of this kind. As can be seen in Table 1, treatment success in the fourth or last follow-up in Tinagrast group is 7 percent more than that of the Neupogen group (74 percent against 67 percent, respectively, $p>0.05$).

Among limitations of this study, besides loss to follow-up, low sample size can also be mentioned. Although this study has been able to achieve the desired sample in the proposal or its protocols, if this clinical trial is considered one with the objective of equal treatment trial, in the current situation, it is essential that the study be assessed about the feasibility of finding a clinically significant difference. However, the important point to be noted is that no clinically significance level is defined for the differences between primary outcomes of the study. This important index is needed to be defined to assess the feasibility of the study in its current condition. In other words, as an example, the following question cannot be answered calaining that the two treatments are of equal strength in treating neutropenia (or fever and neutropenia)

To what extent the differences in the absolute neutrophil count between the two groups can be regarded as a lack of difference in therapeutic effect between the two groups? Does the difference between an average of 200 or 400 mean equal therapeutic effect? Or else?

Finally, it seems that this clinical trial could achieve its intended purpose in the most favorable way possible with regard to its nature. The nature of the present study was to assign random treatment to participants being studied and also to apply double-blinding in patients and physicians who assess the outcomes; this method is known as the most valid of the clinical research design. In addition, due to the repetitive nature of measuring outcomes in four phases ,the fairly regular patient follow-up, the frequency of different outcomes (primary and secondary outcomes), and the different methods and approached applied for analyzing the data, equal or similar effects in the two treatment groups can be accepted. Therefore, Tinagrast can be a logical alternative treatment of cancer in Iran, specifically, if a financial cost and economic burden on patients is added to this comparison. A same study is strongly recommended to be conducted with a greater sample size and longer follow-up (with more time periods), in particular, a phase four clinical trial or Post-marketing Surveillance. This provides the possibility of a credible and scientific judgment in the case of rare or long-term adverse effects associated with Tinagrast as a new drug.

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