

Immunogenicity assessment of tbo-filgrastim in cancer patients receiving chemotherapy

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Aim: This integrated analysis examined the immunogenicity of tbo-filgrastim and its potential clinical impact in three Phase III randomized studies in patients with breast cancer, lung cancer and non-Hodgkin lymphoma receiving chemotherapy. **Results:** Treatment-emergent antidrug antibodies (ADA) occurred in 3/213 (1.4%) breast cancer patients, 2/160 (1.3%) lung cancer patients and 1/63 (1.6%) patients with non-Hodgkin lymphoma. None of the treatment-emergent ADA showed cross-reactivity toward native granulocyte-colony stimulating factors or exhibited neutralizing activity against tbo-filgrastim. Among patients with treatment-emergent ADA, there was no treatment-related hypersensitivity or anaphylaxis and no evidence of loss of clinical efficacy. **Conclusion:** Tbo-filgrastim has demonstrated low immunogenicity in cancer patients receiving chemotherapy and ADA response does not impact safety and efficacy in the patients.

First draft submitted: 24 March 2018; Accepted for publication: 20 June 2018; Published online: 30 July 2018

Keywords: antidrug antibodies • cancer patients • clinical impact analysis • efficacy • filgrastim • safety

Recombinant granulocyte-colony stimulating factors (G-CSF), such as short-acting filgrastim and long-acting pegylated filgrastim are used in standard-of-care treatment for preventing febrile neutropenia in patients receiving myelosuppressive chemotherapy [1]. Choice of G-CSF product for a patient is based on convenience, cost and clinical context [1]. Filgrastim is approved by the US FDA as a prophylaxis for chemotherapy induced neutropenia under the trade name Neupogen[®] [2]. Tbo-filgrastim is a human recombinant G-CSF with a nonglycosylated methionyl group, approved in Europe as a biosimilar to Neupogen[®] in 2008 under the international nonproprietary name filgrastim and was marketed under the trade name Tenvagra[®]. In August 2012, it was approved under a standalone biologic license application in the USA under the brand name Granix[®] for reducing the duration of severe neutropenia in patients with nonmyeloid malignancies receiving myelosuppressive chemotherapy associated with a clinically significant incidence of febrile neutropenia [3]. Tbo-filgrastim has demonstrated similar efficacy and safety to filgrastim (Neupogen[®]) in three multinational, randomized, Phase III trials in patients with breast cancer, lung cancer and non-Hodgkin lymphoma (NHL) receiving chemotherapy [4–6].

Immunogenicity is the propensity of the therapeutic protein product to generate immune responses to itself and to related proteins or to induce immunologically related adverse clinical events [7]. Being a large molecule, a therapeutic protein can trigger antidrug antibody (ADA) responses, including binding antibodies and neutralizing antibodies (NAb) to itself and/or a molecule with similar structure (e.g., endogenous counterpart). Immunogenicity of a biological product is a critical concern for patients, clinicians and regulators [8,9]. Therefore, immunogenicity assessment is required for the development of any biological products. Induction of ADA may have serious consequences including anaphylaxis resulting from hypersensitivity [7,9]. In addition, a biological product with an endogenous counterpart may stimulate generation of antidrug NAb that potentially cross-reacts with and inhibit the endogenous counterpart, resulting in serious adverse effects if the endogenous counterpart serves an essential function.

In clinical studies, filgrastim (Neupogen[®]) exposure was found to elicit the production of ADA against filgrastim in 3% of patients, but no neutralizing activity was detected in these patients [10].

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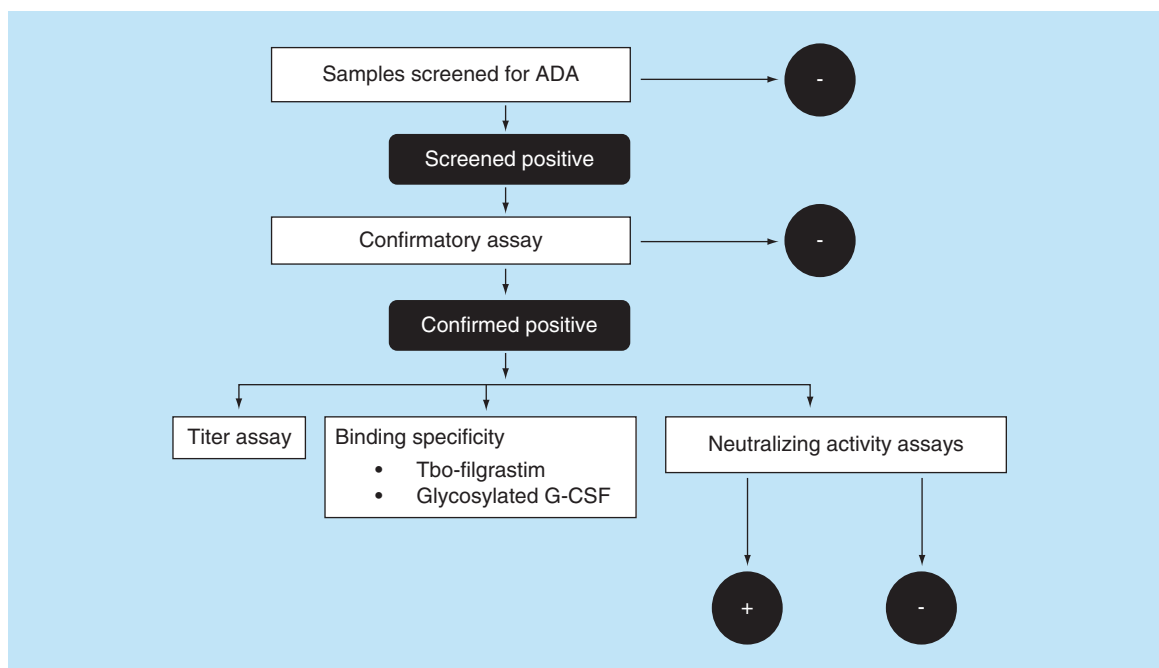


Figure 1. Sequential cascade of immunogenicity assays.

ADA: Antidrug antibodies; G-CSF: Granulocyte-colony stimulating factors.

In this manuscript the immunogenicity of tbo-filgrastim, including the presence of binding antibodies and NAb against tbo-filgrastim and its endogenous counterpart (native G-CSF), was assessed using data from all three Phase III clinical trials in patients with breast cancer, lung cancer and NHL receiving chemotherapy. The potential clinical impact of anti-tbo-filgrastim antibodies on patient safety and efficacy was also evaluated.

Experimental

Study design, patients & treatments

Immunogenicity was assessed using serum samples collected from 436 cancer patients treated with tbo-filgrastim after chemotherapy in three independent Phase III clinical studies [4–6]. The first study was a Phase III, randomized, controlled study that compared tbo-filgrastim with filgrastim in 213 patients with Stage II, III or IV breast cancer receiving docetaxel/doxorubicin chemotherapy [4]. Patients were randomized 2:2:1 to receive equivalent subcutaneous doses of either tbo-filgrastim or filgrastim (both 5 µg/kg/day) or placebo (cycle 1 only) 1 day after chemotherapy for 5–14 days in each of the four cycles. Patients in the placebo arm received tbo-filgrastim after completion of cycle 1, so these patients were exposed to tbo-filgrastim during cycles 2–4. The second study was a Phase III, randomized, controlled, comparative study in 160 patients with small cell or non-small-cell lung cancer requiring platinum-based chemotherapy [6]. Patients received subcutaneous injections of either tbo-filgrastim or filgrastim (both 5 µg/kg/day) 1 day after chemotherapy for 5–14 days in each cycle for up to six cycles. The third study was conducted in 63 patients with aggressive NHL (defined as diffuse large B cell lymphoma, mediastinal large B cell lymphoma, follicular lymphoma Grade III or anaplastic large cell lymphoma) eligible to receive standard chemotherapy [5]. This study had the same design and treatment schedule as the second study conducted in lung cancer patients.

Immunogenicity assays for ADA analysis

A sequential cascade of validated bridging immunoassays was implemented to analyze ADA for tbo-filgrastim (Figure 1). All samples were first analyzed in the screening assay to determine the potential presence of anti-tbo-filgrastim antibodies. Samples that screened positive were subsequently analyzed in the confirmatory assay. When confirmed positive, samples underwent further testing for ADA characterization to determine titer, crossreactivity to the native G-CSF and ADA neutralization activity in separate assays.

Screening assay

In the screening assay, human serum samples and controls (normal pooled human serum with and without rabbit anti-tbo-filgrastim polyclonal positive control antibody at 450 ng/ml and 2500 ng/ml) were incubated overnight at ambient temperature with biotinylated and digoxigenin-conjugated tbo-filgrastim (each at 0.3 µg/ml) to form an antibody antigen complex. The complex was captured on a streptavidin-coated plate and then detected using horseradish peroxidase-labeled anti-DIG antibody (Roche) diluted 1:800 and quantified with a 3,3',5,5'-tetramethylbenzidine substrate (SeraCare, MA, USA). A sample was considered to be screened positive if its absorbance was equal to or greater than the plate cut point. The plate cut point was calculated by multiplying the assay response of the negative control with the population-specific cut-point factor, which was calculated from predose samples from respective patients of each clinical study. Cut-point factors with a false positive rate set at 5% were determined using statistical methodology. Based on predose sample responses, the calculated in-study cut-point factors for breast cancer, lung cancer and NHL were 1.10, 1.03 and 1.04, respectively.

Confirmatory assay

Screened positive samples were confirmed for ADA specificity to tbo-filgrastim in an immunocompetition assay. ADA binding was measured in the absence versus presence of tbo-filgrastim at 1.33 µg/ml. The assay result was expressed as a percentage of signal inhibition in the presence versus absence of tbo-filgrastim. Samples were identified as confirmed positive if the percentage of signal inhibition was greater than or equal to the population-specific confirmatory cut point (% inhibition) for tbo-filgrastim, which was statistically determined based on a 1% false positive rate. In the confirmatory assay, population-specific cut points (% inhibition) for breast cancer, lung cancer and NHL patient populations were 22.5, 28.6 and 22.0%, respectively.

Cross-reactivity characterization assay

To examine potential ADA cross-reactivity with endogenous G-CSF, the confirmed-positive samples were tested with Granocyte (Chugai Pharma, Tokyo, Japan), a glycosylated form of G-CSF mimicking the native G-CSF in an immunocompetition assay. ADA binding was measured in the presence versus absence of Granocyte at 1.33 µg/ml and the assay result was expressed as a percentage of signal inhibition. Samples were identified as cross-reactive to the native G-CSF if the percentage of signal inhibition was greater than or equal to the population-specific cut point (% inhibition) for Granocyte, which was 21.7, 31.5 and 28.9% for breast cancer, lung cancer and NHL, respectively. Like the confirmatory cut point, the cross-reactivity characterization cut point (% inhibition) was also statistically determined based on a 1% false positive rate.

Titer assay

To determine ADA titer for each confirmed sample, a titration curve was generated from assay signal versus sample dilution. ADA titer was calculated as a logarithm of the interpolated dilution factor that resulted in assay signal intersecting at the plate cut point. The log₁₀ titer was reported for samples. The in-study cut-point factors that were determined for the screening assay (1.10 for breast cancer, 1.03 for lung cancer and 1.04 for NHL) were also used for the titer assay to calculate plate cut point.

NAb analysis

ADA neutralizing activity was assessed in a cell-based proliferation assay that detects the ability of ADA to inhibit tbo-filgrastim- or Granocyte-stimulated proliferation of NFS-60 cells *in vitro*. In this analysis, assay controls (pooled normal human serum with and without neutralizing goat antihuman G-CSF polyclonal antibody (R&D Systems, MN, USA) at 1000 ng/ml and human serum test samples were added to a fixed concentration of tbo-filgrastim (28 pg/ml) or Granocyte (28 pg/ml) at 50 µl /well and then mixed with NFS-60 cells (1.0×10^4 cells/well) at 50 µl/well. The cells were allowed to proliferate at 37°C with 5% CO₂ and 95% air for 40–50 h. NAb directed against tbo-filgrastim and Granocyte bind to tbo-filgrastim and Granocyte, respectively and inhibit NFS-60 cell proliferation that is measured using a chemiluminescence technique (CellTiter-Glo®, Promega, WI, USA). Determination of NAb positivity is based on the reduction of tbo-filgrastim- or Granocyte-induced cell proliferation by a test serum sample relative to that of the negative control sample (pooled normal human serum). To ensure that specific assay responses were reported, each sample was tested under four conditions (i.e., without inducer, with tbo-filgrastim inducer, with Granocyte inducer and with mIL-3 inducer at 50 pg/ml). Samples were identified as NAb positive, if the proliferation induced by tbo-filgrastim or Granocyte was inhibited (i.e., having an assay

Table 1. Antidrug antibody titer, cross-reactivity and neutralizing antibody analysis: antidrug antibody-positive samples from patients with breast cancer.

Cohort	ADA (titer [†])/competitor/NAb	Time-point						
		Predose	C1D1	C2D1	C3D1	C4D1	D85	D180
Placebo	ADA (titer)	Neg	Neg	–	Neg	Pos (0.120)	Neg	–
	glycoG-CSF	–	–	–	–	Neg	–	–
	NAb	–	–	–	–	Neg	–	–
Tbo-filgrastim (1)	ADA (titer)	Neg	Neg	Neg	–	–	Neg	Pos (0.199)
	glycoG-CSF	–	–	–	–	–	–	Neg
	NAb	–	–	–	–	–	–	Neg
Tbo-filgrastim (2)	ADA (titer)	–	Neg	Neg	Neg	Pos (<0.00) [‡]	Neg	Neg
	glycoG-CSF	–	–	–	–	Neg	–	–
	NAb	–	–	–	–	Neg	–	–

ADA: Antidrug antibody; C1D1: Cycle 1 day 1; C2D1: Cycle 2 day 1; C3D1: Cycle 3 day 1; C4D1: Cycle 4 day 1; D85: Day 85; D180: Day 180; glycoG-CSF: Glycosylated granulocyte colony stimulating factor; NAb: Neutralizing antibody; Neg: Negative by screen or confirm; Pos: Confirmed-positive sample.
[†]Titer values are in log₁₀ scale and were determined for confirmed-positive samples only.
[‡]Negative titer with signal below cut point.

response less than or equal to the cut point), while nonspecific inhibition was not observed (i.e., the mIL-3-induced proliferation was greater than the cut point in the presence of a test sample). If mIL-3-induced cell proliferation was inhibited by a test sample (i.e., an assay response less than or equal to the mIL-3 inducer cut point), the inhibition by the sample would be declared nonspecific inhibition rather than neutralizing activity to tbo-filgrastim or Granocyte. Samples were identified as samples with nonspecifically inducing cell proliferation in the presence of the test samples if proliferation without the inducer was observed. Cut points with a 1% false positive rate were determined for conditions with tbo-filgrastim, Granocyte or mIL-3 inducers, and under conditions without an inducer by statistical methods using commercially available serum samples from treatment-naïve patients with breast cancer, lung cancer and NHL. The cut points were 0.807 for tbo-filgrastim inducer, 0.894 for Granocyte inducer, 0.836 for mIL-3 inducer and 10.308 for no inducer. To rule out potential interference of hIL-6 on the NAb assay, the Quantikine ELISA hIL-6 Immunoassay Kit (R&D Systems) was used to determine hIL-6 levels.

Clinical impact analyses of ADA

To evaluate potential ADA impact on patient safety, a review of medical records was conducted for all patients with confirmed-positive ADA samples for evidence of potential allergic reactions. The following conditions were screened: angioneurotic edema, allergic dermatitis, drug hypersensitivity, hypersensitivity, rash and rash pruritic and urticarial. Potential loss of efficacy in ADA-positive patients was also evaluated based on observed incidences of febrile neutropenia (defined as a body temperature of above 38.5°C for more than 1 h and an absolute neutrophil count below $0.5 \times 10^9/\text{l}$, both measured on the same day).

Results

A total of 436 cancer patients who received tbo-filgrastim from the three Phase III studies were analyzed for immune response to the study drug. These included 213 patients with breast cancer (72 in the placebo/tbo-filgrastim arm, 141 in the tbo-filgrastim arm), 160 patients with lung cancer and 63 patients with NHL.

Immunogenicity of tbo-filgrastim in patients with breast cancer

Out of 213 patients with breast cancer, three patients, each with one postdose sample, were confirmed positive for ADA (Table 1), representing immunogenicity incidence of 1.4%. None of the three patients was positive for ADA at the predose time point. One of the three patients was in the placebo arm of the study in the first cycle and received tbo-filgrastim in cycles 2–4. The ADA-positive sample occurred on cycle 4, after two cycles of tbo-filgrastim treatment in this patient. The other two patients were in the tbo-filgrastim arm of the study. ADA positivity was observed in cycle 4 in one patient and on day 180 of the follow-up period after cycle 4 in the other patient. None of the three patients had ADA that cross-reacted with the native (glycosylated) G-CSF or demonstrated NAb activity. The hIL-6 levels were determined for all three confirmed-positive samples, and the results were all below 1000 pg/ml, confirming no interference by hIL-6 in the test samples during NAb testing (Table 2). The ADA titer

Table 2. hIL-6 results for confirmed-positive samples in cancer patients.

Study	Treatment	Visit	hIL-6 [†] (pg/ml)
Breast	Placebo	C4D1	10
	Tbo-filgrastim (1)	D180	6
	Tbo-filgrastim (2)	C4D1	4
Lung	Tbo-filgrastim (1)	C6D1	4
	Tbo-filgrastim (2)	C1D1	11
	Tbo-filgrastim (3)	C3D1	238
NHL	Tbo-filgrastim	C3D1	3

hIL-6: Human interleukin 6; C1D1: Cycle 1 day 1; C3D1: Cycle 3 day 1; C4D1: Cycle 4 day 1; C6D1: Cycle 6 day 1; D180: Day 180; NHL: Non-Hodgkin lymphoma.
[†]All samples <1000 pg/ml of hIL-6.

Table 3. Antidrug antibody titer, cross-reactivity and neutralizing antibody analysis: antidrug antibody-positive samples from patients with lung cancer.

Patient	ADA (titer [†])/competitor/NAb	Time-point									
		Predose	C1D1	C2D1	C3D1	C4D1	C5D1	C6D1	D127	D169	D180
1	ADA (titer)	–	Neg	Neg	Neg	Neg	Neg	Pos (<0.00) [‡]	Neg	–	Neg
	glycoG-CSF	–	–	–	–	–	–	Neg	–	–	–
	NAb	–	–	–	–	–	–	Neg	–	–	–
2	ADA (titer)	Neg	Pos (<0.00) [‡]	Neg	Neg	Neg	Neg	Neg	–	Neg	–
	glycoG-CSF	–	Neg	–	–	–	–	–	–	–	–
	NAb	–	Pos [§]	–	–	–	–	–	–	–	–
3	ADA (titer)	Neg	–	Neg	Pos (NR) [¶]	Neg	–	–	Neg	–	–
	glycoG-CSF	–	–	–	Neg	–	–	–	–	–	–
	NAb	–	–	–	Neg [#]	–	–	–	–	–	–

ADA: Antidrug antibody; C1D1: Cycle 1 day 1; C2D1: Cycle 2 day 1; C3D1: Cycle 3 day 1; C4D1: Cycle 4 day 1; C5D1: Cycle 5 day 1; C6D1: Cycle 6 day 1; D127: Day 127; D169: Day 169; D180, Day 180; glycoG-CSF: Glycosylated granulocyte-colony stimulating factor; NAb: Neutralizing antibody; Neg: Negative by screen or confirm; Pos: Confirmed-positive sample.
[†]Titer values are in log₁₀ scale and were determined for confirmed-positive samples only.
[‡]Negative titer with signal below cut point.
[§]NAb positive for tbo-filgrastim only.
[¶]NR: Not reportable because of indeterminate titer.
[#]Nonspecific inhibition.

was low for all three positive samples, ranging from below the level of detection to 0.199 in log₁₀ scale.

Immunogenicity of Tbo-filgrastim in patients with lung cancer

Out of 160 patients with lung cancer, three patients, each with one sample, were confirmed positive for ADA. ADA titers of all three samples were too low to be determined (Table 3). One sample was confirmed positive for ADA on day 1 of chemotherapy on cycle 1, which was 24 h prior to the first dose of tbo-filgrastim. This sample also demonstrated neutralizing activity toward tbo-filgrastim, but was considered a pre-existing response that was unrelated to tbo-filgrastim because of the timing. The other two confirmed-positive samples both occurred after tbo-filgrastim administration. One of these samples was confirmed positive on cycle 3 and the other on cycle 6, representing an immunogenicity incidence of 1.3% in lung cancer patients. There was no NAb activity associated with these two samples. None of the three samples had ADA that cross-reacted with native G-CSF. The hIL-6 levels were less than 1000 pg/ml for all three samples, confirming no interference by hIL-6 during NAb testing (Table 2).

Immunogenicity of Tbo-filgrastim in patients with NHL

Among 63 patients with NHL, only one patient was confirmed positive for ADA (Table 4). The positive sample occurred post-treatment in cycle 3, but the ADA titer was too low to determine. There was no cross-reactivity to native G-CSF or neutralizing activity associated with this sample. As only one of 63 patients developed an ADA response, this represented an immunogenicity incidence of 1.6% in patients with NHL. There was no hIL-6 interference to NAb testing with this sample (the hIL-6 level was below 1000 pg/ml) as is shown in Table 2.

Table 4. Antidrug antibody titer, cross-reactivity and neutralizing antibody analysis: antidrug antibody-positive samples from patients with non-Hodgkin lymphoma.

Cohort	ADA (titer [†])/competitor/NAb	Time-point							
		Predose	C2D1	C3D1	C4D1	C5D1	C6D1	D127	D180
Tbo-filgrastim	ADA (titer)	Neg	Neg	Pos (<0.00) [‡]	Neg	Neg	Neg	Neg	Neg
	glycoG-CSF	–	–	Neg	–	–	–	–	–
	NAb	–	–	Neg	–	–	–	–	–

ADA: Antidrug antibody; C2D1: Cycle 2 day 1; C3D1: Cycle 3 day 1; C4D1: Cycle 4 day 1; C5D1: Cycle 5 day 1; C6D1: Cycle 6 day 1; D127: Day 127; D180: Day 180; glycoG-CSF: Glycosylated granulocyte-colony stimulating factor; NAb: Neutralizing antibody; Neg: Negative by screen or confirm; NHL: Non-Hodgkin lymphoma; Pos: Confirmed-positive sample.
[†]Titer values are in log₁₀ scale and were determined for confirmed-positive samples only.
[‡]Negative titer with signal below cut point.

Clinical impact

Individual patient records from the three cancer studies were reviewed for the seven patients with a confirmed-positive ADA sample to identify potential allergic reactions. None of the seven patients experienced any of the potential allergic reactions. Furthermore, none of these patients demonstrated loss of tbo-filgrastim efficacy based on the rates of febrile neutropenia during the study or suffered from febrile neutropenia during chemotherapy treatment.

Discussion

Recombinant G-CSF products are the current standard of care for the prevention of chemotherapy-induced neutropenia in cancer patients. However, only a limited number of options are available (i.e., filgrastim and its biosimilars or pegylated filgrastim). The addition of tbo-filgrastim to the therapeutic armamentarium would represent a meaningful public health benefit for cancer patients.

An important aspect of biological product development is clinical immunogenicity assessment because ADA induced by the treatment may impact safety or efficacy. The cause of ADA production is unclear, and many mechanisms have been proposed, some of which include the presence and/or exposure of epitopes, reactions against drug adjuvants, the influence of concomitant diseases, genetic predisposition of the patient and previous exposure to a similar compound [8]. Immunogenicity of a therapeutic protein product can vary in different patient populations [7]. Thus, it is imperative that each biological product be evaluated for potential immunogenicity to ascertain product safety in each indicated patient population.

The objective of this analysis was to evaluate the immunogenicity of tbo-filgrastim in patients receiving chemotherapy for solid and hematologic malignancies. For this purpose, a tiered sample analysis approach was implemented to detect and characterize ADA responses to subcutaneous administration of tbo-filgrastim in three cancer patient populations. A bridging assay was used, that is capable of detecting all isotypes of ADA [11] and the assay was validated according to industry recommendations [12] and FDA guidance [13] for required sensitivity, specificity, precision and robustness. To ensure that low-titer ADA was detected, population-specific cut-point factors were calculated using 5% false positive rate and based on predose samples from each specific study. ADA characterization was thoroughly conducted for determination of titer, cross-reactivity to the native G-CSF and neutralizing activity of ADA. A cell-based assay was used for NAb determination and this assay was developed and validated based on industry recommendations [14,15]. Since the cell line (NFS-60) could nonspecifically respond to matrix components, each sample was analyzed under multiple conditions (i.e., without inducer, with tbo-filgrastim inducer, with Granocyte inducer and with mIL-3). In addition, as NFS-60 cell line can proliferate in response to hIL-6 potentially present in the test samples, further analysis was conducted to examine potential interference of assay results. It was experimentally determined that hIL-6 at or above 1000 pg/ml could induce NFS-60 cell proliferation on top of the tbo-filgrastim or Granocyte inducer, potentially resulting in failure to detect NAb activity in such samples containing high levels of hIL-6. Therefore, the levels of hIL-6 were quantified for all samples during NAb analysis to identify samples with potential hIL-6 interference. A hIL-6 NAb would be added to samples with hIL-6 concentration greater than 1000 pg/ml to mitigate hIL-6 interference in the NAb assay and samples would be re-analyzed. However, no such action was taken as hIL-6 levels determined in all test samples were below 1000 pg/ml (Table 2). These extensive testing procedures ensure reliability of NAb analysis [14].

In these studies, the incidence of treatment-emergent ADA was consistently low for all three patient populations, with immunogenicity incidences being approximately 1.4, 1.3 and 1.6% for patients with breast cancer, lung

cancer and NHL, respectively. The titers for all positive samples were also low, ranging from undetectable to 0.199 in $\log_{10}$ scale. There was no cross-reactivity with endogenous G-CSF associated with treatment-emergent ADA and all confirmed-positive samples after tbo-filgrastim treatment did not exhibit neutralizing activity against both tbo-filgrastim and native G-CSF. These results eliminate a major safety concern for immunogenicity of a biological product with an endogenous counterpart, which could indicate an autoimmune response and breakage of immunologic tolerance [8]. Among the seven tbo-filgrastim-exposed patients with a positive ADA sample, no apparent impact on the incidence of febrile neutropenia was observed that may be correlated with ADA positivity in these patients. A review of patient records found no evidence of potential allergic reactions and no loss of efficacy during the studies. These results further confirm the safety of tbo-filgrastim in these patients.

The low incidence of confirmed, treatment-emergent ADA and lack of NAb response following tbo-filgrastim treatment are consistent with the known immunogenicity profile of filgrastim in cancer patients, for which the reported ADA rate was 3% [10]. However, given the potential differences in clinical study design, ADA methodologies, and assay sensitivity and specificity, the ability to compare immunogenicity rates between products is limited. One limitation of this study was that immunogenicity samples after filgrastim treatment, which was included as a comparator in all three Phase III studies, were not analyzed for comparison because tbo-filgrastim was submitted as a standalone biologic license application. Availability of immunogenicity data from these direct head-to-head studies comparing tbo-filgrastim with filgrastim would be very helpful to evaluate relative immunogenicity of these two products.

Cancer patients receiving a chemotherapy may have a compromised immune system that is less responsive to immunogen. So the low immunogenicity of tbo-filgrastim may not reflect the immunogenicity of tbo-filgrastim in other populations, especially normal population. However, the immunogenicity data of tbo-filgrastim in healthy volunteers is not available for comparison due to a lack of reliable immunogenicity method for sample analysis in early Phase I trials.

Overall, tbo-filgrastim has demonstrated low immunogenicity in cancer patients, and ADA responses were not associated with adverse events or loss of efficacy.

Conclusion

This integrated analysis examined the immunogenicity of tbo-filgrastim and its potential clinical impact in cancer patients. Our analyses showed that tbo-filgrastim had low immunogenic activity and no treatment-related hypersensitivity/anaphylaxis or loss of clinical efficacy.

Future perspective

Comparative immunogenicity data is an integrated element of biosimilarity determination. As more and more biosimilar or follow-on biologic products move into development pipelines, it is interesting for the scientific community to see how comparable immunogenicity profiles will be between biosimilar and reference products and how the differences, if any, impact clinical outcomes in a late-stage head-to-head study.

Financial & competing interests disclosure

The authors are employees of Teva Pharmaceuticals. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Aim

- Immunogenicity assessment is required for the development of a biological product.
- Tbo-filgrastim is a recombinant human granulocyte-colony stimulating factor (G-CSF) approved in USA as a standalone biologics.

Experimental

- Immunogenicity was assessed using serum samples collected from patients treated with tbo-filgrastim after chemotherapy in three independent Phase III clinical studies in patients with breast cancer, lung cancer and non-Hodgkin lymphoma.
- A sequential cascade of validated bridging immunoassays, including screening, confirmatory and characterization methods was implemented to analyze antidrug antibodies (ADA) against tbo-filgrastim.

Results & discussion

- Treatment-emergent ADA occurred in 3/213 (1.4%) breast cancer patients, 2/160 (1.3%) lung cancer patients and 1/63 (1.6%) patients with non-Hodgkin lymphoma.
- There was no cross-reactivity of ADA with native G-CSF, and ADA exhibited no neutralizing activity against both tbo-filgrastim and native G-CSF.
- There was no treatment-related hypersensitivity or anaphylaxis and no evidence of loss of clinical efficacy of tbo-filgrastim in these trials.

Conclusion

- Tbo-filgrastim has low immunogenicity in cancer patients receiving chemotherapy.
- ADA responses against tbo-filgrastim do not impact safety and efficacy in the patients.

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