CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

BLA 125103/0

Immunogenicity Review

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Date: final rev ver/ 17 Nov 04
From: Ralph M Bernstein
To: File, **STN 125103**
Through: Elizabeth Shores, Amy Rosenberg.
CC: Kurt Stromberg, Lead CMC reviewer STN **125103.**
Re: Review of STN 125103/Palifermin Immunogenicity Assay, Neutralization assay, labeling comments.

Immunogenicity Assay
Overview:
Amgen has submitted a package within the BLA submission for STN 125103 that addresses the validation of techniques developed to monitor the development of antibodies in patients treated with Palifermin/rHuKGF. The method, Amgen number [ ] entitled "Immunoassay to Detect Antibodies in rHuKGF in Human Serum using the [ ] is a bridging assay (herein referred to as the ECL assay) which utilizes two species of recombinant product (Palifermin/KGF) that is both labeled with a detection agent [ ] and — antibody (patient sera) is preincubated with both species in a 96 well format plate, the wells of which are [ ] (see **Figure 1**, attached at end of document). If there is reactive antibody present in the wells, a bridge is formed [ ] Palifermin and the detection agent bound Palifermin. The addition of a substrate [ ] allows detection and quantification of the antibodies.

Assay components:
Negative control: pooled normal human serum (pNHS), diluted to — in assay diluent buffer, is used as the assay negative control. New lots of pNHS are tested against the current lot, and only lots with less than a — variance in raw ECL signal are used.

Positive control: affinity purified rabbit anti rHuKGF is diluted into neat pNHS to 100ng/ml, then diluted to — in assay diluent. New lots of anti-KGF will be tested against the current lot to ensure a signal above the threshold (see threshold, below).

[ ] rHuKGF: — rHuKGF is diluted into assay diluent to — g/ml, aliquots of which are stored at — C or colder, and expire after one year post creation date. — labeling is performed by Amgen's analytical sciences per SOP [ ] Binding of — HuKGF will be compared to that of the non labeled molecule using a [ ] instrument as described in validation report [ ], and the component will not be used if the two components vary more than — in binding rates.

[ ] rHuGKF: — rHuGKF is diluted into assay diluent to — ng/ml, aliquots of which are stored at — C or colder, and expire after one year post creation date. — labeling is performed by Amgen's analytical sciences per SOP [ ] Binding of — pHuKGF will be compared to that of the non labeled molecule using a [ ]

instrument as described in validation report [redacted] and the component will not be used if the two components vary more than [redacted] in binding rates.

Assay development:

Threshold: the threshold (or cutpoint) for the ECL assay was developed by analyzing the activity of 99 individual pre-treatment patient serum samples in the ECL assay: these samples generate a raw ECL value. This value was divided by the raw ECL value of pNHS also analyzed in the ECL; this value is called the signal to noise ratio, or S/N. All sample reactivity values are expressed in this manner: raw ECL of sample divided by raw ECL of a pNHS control. The mean S/N of these samples was analyzed and the upper bound of a one sided 95% prediction interval (the mean S/N plus 1.645 standard deviations (SD)) was determined to have a numerical value of 1.21. Amgen states that after statistical analysis of the distribution of the S/N values, that the distribution is normal, with no outliers (an outlier being defined as 3 SD from the mean). This is the assay cutpoint. This value allows for a [redacted] false positive rate, to minimize the occurrence of false negatives.

Assay sensitivity: the sensitivity of the assay was determined using affinity purified (AP) rabbit polyclonal anti rHuKGF. The sensitivity is the amount of antibody that can be detected in the assay above the threshold S/N value of 1.21. Antibody concentrations ranging from [redacted] ng/ml spiked into 100% pNHS demonstrated that [redacted] ng/ml exhibited a S/N value of [redacted] demonstrating that the assay sensitivity is [redacted] ng/ml. See **Table 1**, attached at end of document.

Quantitiation limit: to determine the about of antibody in a given sample that could be reliably detected using the ECL assay, Amgen spiked individual serum samples from patients with hematological malignancies with 25, 50, and 100ng/ml of the rabbit AP anti-rHuKGF antibody. A statistical analysis of the S/N values generated demonstrated that assay variability allowed for the reliable detection of [redacted] ng/ml of antibody. [redacted] ng/ml is the assay's limit of quantification (LOQ); this value has an S/N of [redacted]

Assay specificity:

1) Assay specificity was demonstrated using three approaches; the first demonstrated that while soluble rHuKGF preincubated with specific antisera inhibited detection of anti KGF antibodies, unlabled FGF-10 similarly preincubated showed little or no inhibition of signal. In contrast, unlabled FGF-7 (full length KGF) reduced the ECL signal when similarly preincubated, albeit at relatively high concentrations (e.g., 400 and 800 ng/ml, see **Figure 2.** attached at the end of this document). In the BLA, (section 3-2-r) Amgen states that the dose dependant properties of rHuKGF, the FGF-10 non result, and the relatively uninhibitory result with FGF-7 demonstrates the specificity of the assay for KGF, and not other KGF family members. If anti KGF antibodies were to develop in a patient, this crossreactivity with FGF family members may be a concern for clinicians, but the clinical significance is not understood, and would be hard to predict, as FGF is a large family with some sequence similarities and some limited overlapping functions.

2) The second approach utilized unrelated antibodies, and demonstrated that they, in contract with anti rHuKGF, exhibited signals well below the threshold value of 1.21. This demonstrates that the detection of KGF is due to the specificity of anti KGF antibodies and not background or anomalous antibody binding.

3) Immunodepletion: this test is used to confirm the specificity of antisera that generate a signal above the threshold of 1.21. Amgen determined the concentration of rHuKGF that can reproducibly and significantly reduce the ECL values of specific antibody binding; this was accomplished by spiking pNHS with 100, 800, and 2400ng/ml of anti rHuKGF that was preincubated with increasing concentrations of rHuKGF. Amgen determined that 800ng/ml was effective in reducing wide antibody ranges by — or greater. (In all spiked antibody concentrations, Amgen determined that — ıg/ml of rHuKGF reduced the relative signal by greater than —).

Qualification and interference of labeling of rHuKGF assay components: a concern regarding the labeling of rHuKGF for use in the assay, is that the modification of the molecule might reduce antisera binding/recognition, or abrogate it completely. To address this issue, Amgen immobilized rHuKGF, — rHuKGF, and — rHuKGF onto a [ ] and the binding of AP-rabbit polyclonal anti rHuKGF serum was evaluated and determined to have comparable affinities. This analysis suggests that rHuKGF conjugation may not significantly affect the monitoring of anti rHuKGF antibodies in patient samples.

Precision: Amgen demonstrated the intra assay precision (the reproducibility of the assay results regarding an individual sample) of the ECL assay to have a CV of [ ] %. The inter assay precision (the measure of reproducibility over several days regarding an individual samples) to have a CV of —

Freeze thaw stability: Amgen addressed the issue of sample stability after [ ] freeze thaws, by spiking 100 and 800ng/ml of anti rHuKGF antisera into pNHS samples and subjected these samples to — freeze thaw cycles; these experiments demonstrated that 100ng/ml spiked antibody yielded results ranging from [ ] recovery with a CV of [ ]; 800ng/ml spiked antibody yielded results ranging from [ ] % with a CV.

Assay analysis:

Sample positivity: samples are considered reactive if the S/N is greater than 1.21 (the threshold). Samples with S/N values greater than [ ] (the LOQ) are reanalyzed with the immunodepletion test; samples demonstrating a greater than 50% reduction in signal were then designated positive. Patients are considered positive for antibody development if :

Positive and negative reporting criteria: if a sample's S/N is lower than the threshold of 1.21, the sample is termed negative; if a sample's S/N is within the range of 1.21 to [ ], the sample is positive below the quantifiable limit; if a sample's S/N is equal to or greater than — and demonstrates a 50% or greater loss of reactivity in the immunodepletion

test, the sample is termed positive; if the sample demonstrates less than — reduction in reactivity in the immunodepletion, it is termed negative for anti- rHuGKF antibodies.

Post treatment reporting criteria: if a post treatment sample's S/N is greater than 1.21, and its post treatment to pre treatment ratio is greater than the quantitation threshold of — if the sample demonstrates 50% or greater decrease in ECL signal in the immunodepletion test, then they are positive for post treatment development of anti KGF antibodies. Samples that have less than a 50% decrease in their ECL S/N signal in the immunodepletion test are termed negative.

Clinical trial immunogenicity results:
Amgen analyzed 964 patients using the ECL assay; 12 patients (~1%) tested positive above the assay threshold, i.e., 1.21, and were termed reactive. Two of these patients, in studies 980231 and 20000162, were above the LOQ of — and were tested in the immunodepletion assay; these samples did not have a reduced ECL value in the immunodepletion assay (personal communication 10 Nov 04, Dr. Gene Koren, see telecon notes, R Bernstein 10Nov04) and were suspected by Amgen to be "sticky" or non-specific binders. As per Amgens protocol, all 12 subjects above the threshold value of 1.21 were tested in the bioassay/neautralization assay; none of these subjects demonstrated neutralizing activity as assessed by the bioassay.

Conclusion:
Amgen has validated an anti rHuKGF detection assay that is able to reliably and reproducibly detect antibodies directed to rHuKGF to — g/ml sensitivity. It is the opinion of this reviewer that this assay is a reasonably acceptable assay, and will allow the detection of anti rHuKGF antibodies in patient sera. Labeling should reflect the possibility of antisera generated to KGF/Palifermin being crossreactive with FGF family members, and the potential clinical implications that may be associated with such a possibility.

Neutralization assay
Overview: Amgen has submitted a package within the BLA submission for STN 125103 that addresses the validation of techniques developed to monitor the ability of antibodies that are specific for rHuKGF /KGF to neutralize soluble KGF. The validation package, Amgen numbers [ ], entitled "Validation of the Bioassay for the detection of Neutralizing antibodies against recombinant human keratinocyte growth factor (rHuKGF) in 1% human serum." and "Re-establishment of thresholds for the screening assay (Ratio 1), the pre-dose to post-dose ratio (ratio 2), and the specificity assay (ratio 3) for the bioassay detecting neutralizing antibodies against tHuKGF." are included in the BLA along with the Amgen analytical procedure [ ] 'A bioassay for the detection of neutralizing antibodies to rHuKGF in human serum." This assay, adapted from the KGF potency assay, uses a KGF/EPO receptor chimera that is stabily transfected in a murine 32D cell line. These cells, when treated with KGF, respond in a dose dependant manner. When antibodies specific for KGF are present, it interferes with the ability of the KGF to bind the KGF receptors, thereby inhibiting

proliferation [ ] The sensitivity was validated to [ ] g/ml of anti KGF antisera in 100% human serum.

Assay validation:

Validation of the rHuKGF dose response using KECA cells:

[

]

Briefly, the three thresholds are currently set at ratio [
]

In conclusion: Amgen has developed a neutralization assay which, while not optimal, has acceptable specificity and reproducibility for a neutralization bioassay. Amgen has set the cutpoints for this assay at the — false positive rate. This should be acceptable in the case of a neutralizing bioassay, due to the similar ranges of [ ] %. All patients that have been tested in the neutralization/ bioassay have been deemed negative.

Labeling: I recommend that the following text should be added to the package insert:

"Immunogenicity

As with all therapeutic proteins, there is a potential for immunogenicity. The clinical significance of antibodies to [TRADE NAME™] is unknown, but could include lessened activity, and/or cross reactivity with FGF family members.

A sensitive electrochemiluminescence-based binding assay was performed on post-treatment sera from 645 subjects treated with [TRADE NAME™] in clinical studies.

The incidence of antibody positivity is highly dependent on the specific assay and its sensitivity. Additionally, the observed incidence of antibody positivity in an assay may be influenced by several factors including sample handling, timing of sample collection, concomitant medications and underlying disease. For these reasons, comparison of the incidence of antibodies to [TRADE NAME™] with the incidence of antibodies to other products may be misleading. "

Figures/Attachments.

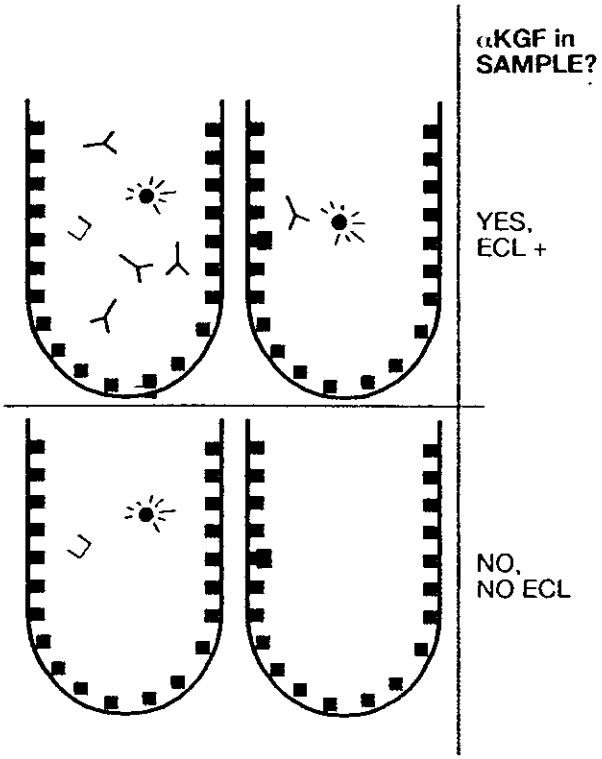

Figure 1. Cartoon schematic demonstrating the detection method of Amgen's anti Palifermin [ECL] assay. In this cartoon, yellow indicates rHuPalifermin, red is the ECL signal generated by the [ ], green squares represent [ ] and green "U"s represent [ ]

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On Original

Table 2. ECL Values and S/N Ratios
In Serum Samples Spiked with anti-rHuKGF Antibodies

Antibody concentration (ng/mL)	ECL 1	ECL 2	Mean ECL	SD	%CV between replicates	S/N
3200			67359 50	4430 02		
1600			31528.50	1540 79		
800			14452.50	751 65		
400			6875 50	181 73		
200			3631.00	76 37		
100			2024 00	76.37		
50			1287.00	43.84		
25			919 50	51.62		
10			720 50	17.68		
Neg Control			570 00	55 15		

Table 2: pNHS spiked with rabbit polyclonal antibody concentrations from 10 to 3200 ng/mL. The Signal/Noise values are calculated by dividing the Mean ECL of the spiked serum sample by the Mean ECL of the Negative Control (unspiked pNHS)

Table 1. Raw data from experiments establishing the sensitivity of the ECL anti-Palifermin assay.

Figure 2. Inhibition of Signal to Noise Ratio
by rHuKGF, FGF-7, and FGF-10

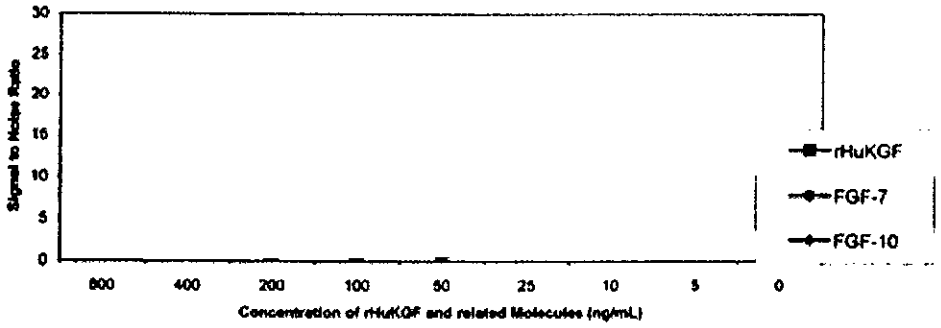

Figure 2: pNHS spiked with 800 ng/mL of anti-rHuKGF and incubated with varying concentrations of rHuKGF, FGF-7 and FGF-10. Graphical presentation of data shown in Table 6

Figure 2. ECL result of specific anti-KGF sera preincubated with KGF, FGF-7 or FGF-10 to demonstrate specificity of potential anti KGF antibodies. Notably, at relatively high concentrations, FGF-7 inhibits the ECL assay, indicating that there is some crossreactivity of anti-KGF antibodies with FGF family members.