PRODUCTION OF RECOMBINANT HUMAN G-CSF: A TECHNO-ECONOMIC ANALYSIS

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Abstract: Filgrastim is an important protein that treats neutropenia which has significant clinical applications including a possible cure against cancer. However, despite its significant medical benefits, the large scale production of Filgrastim is still rare and is reported only by a handful of companies throughout the world. We present a techno-economic feasibility analysis for a recombinant human analog of Filgrastim production on a pilot scale that can be emulated for industrial scale production. The current article provides a general business overview with considerable emphasis on a pilot scale venture highlighting its techno economic analysis and competitive advantages.

Keywords: Granulocyte Colony Stimulating Factor, Filgrastim, Neutropenia, Haematopoiesis.

1. INTRODUCTION

In humans, the synthesis of early blood cells takes place in the bone marrow which is controlled by specific factors called Granulocyte Colony Stimulating Factors or G-CSF (Welte et al., 1996). Filgrastim is a recombinant methionyl human granulocyte colony-stimulating factor (r-met HuG-CSF) produced by inserting the human Granulocyte Colony-Stimulating Factor gene into Escherichia coli (E coli) bacteria and expressing in a suitable host (Welte et al., 1996). Filgrastim is 175 amino acid long with two intrachain disulfide bonds (Hill et al., 1993) and has a molecular weight of 18.8 kiloDaltons or kDa. The amino acid sequence of the protein is similar to that predicted from the analysis of human DNA sequence. However, this does not include the fact that the addition of an N-terminal methionine is important for expression in E coli. Human G-CSF is also a glycoprotein which is O-glycosylated at Thr133 (Oheda et al., 1990). This post-translational event is absent in recombinant human G-CSF produced in E. coli (Souza et al., 1986; Oheda et al., 1990).

Filgrastim contributes to the body’s major defense mechanism against infections by acting on neutrophils. Earlier, it was used as an accessory to chemotherapy for neutropenia (decrease in the neutrophil count in blood). Since its administration, people have been benefitted with the advent of cancer declining progressively (Welte et al., 1996; Hartung et al., 1998). It has been approved throughout the world for treating myelosuppression after bone marrow transplantation, severe chronic neutropenia (SCN), acute leukemia, myelodysplastic syndromes (MDS) and mobilization of peripheral blood progenitor cells (PBPCs) for transplantation (Welte et al., 1996). The use of Filgrastim has also benefited patients who are immunocompromised.

The purification and molecular cloning of Filgrastim (Welte et al., 1985; Platzer et al., 1986; Souza et al., 1986; Nagata et al., 1986; Vanz et al., 2008; Jin et al., 2011) have been reported along with significant clinical trials (Bronchud et al., 1987; Gabrilove et al., 1988; Mortsyn et al., 1988; Ganser and Karthaus, 1996) in recent and earlier studies. GCSF can be produced in large scale and can be commercially synthesized from other heterologous hosts like yeasts (Saiedinia et al., 2008; Apte-Deshpande et al., 2009; Chien, 2010) and mammalian cells (Okabe et al., 1982; Larsen et al., 1990; Safarians et al., 1997) that generate a glycosylated G-CSF as indicated from these studies. However, despite its significant medical benefits, industrial scale production

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of Filgrastim is in practice in only a handful of companies throughout the world. The current article is a techno-economic feasibility analysis for Filgrastim production on a pilot scale that can be emulated for industrial scale production. In Section 2, we discuss a business overview with special emphasis on how to start a new venture. Sections 3 and 4 deal with the market scenario and competitive advantages respectively. Finally, we conclude the study with a financial overview in Section 5, providing a definitive prospect for the business venture.

2. A BUSINESS OVERVIEW: HOW TO START A NEW VENTURE?

2.1 Existing Manufacturing Method in Industries for Filgrastim

To start a new venture, we must first consider the existing manufacturing methods for Filgrastim production in laboratory scale (Herman et al., 2002). Filgrastim is generally prepared from the recombinant N-methionyl G-CSF protein which is expressed by transforming E. coli bacteria that contains the gene sequence for human G-CSF. After harvesting and lysis of the cultured cells, the extracted product is oxidized in its native state. This is followed by several chromatographic and filtration steps. It is then formulated in a special acetate buffer. The active pharmaceutical substance (APS) of the recombinant human granulocyte colony-stimulating factor can also be produced as inclusion bodies (IB). Methods of strain cultivation, IB isolation and downstream processing including quality control have been reported (Herman et al., 2002). The important thing is that though G-CSF is a well characterized molecule, we need to understand its primary, secondary, and tertiary structures for gaining insight into purification, folding, and formulation.

2.2 Materials Required for a Pilot Scale Production of Filgrastim

Human G-CSF analogs (previously prepared) : as raw materials; Crystallization techniques; a high performance Computer program (to observe 3D structure); M13 vectors (conventional vectors used for any site directed mutagenesis); Suitable restriction enzymes (depending on the organism); a suitable plasmid vector; a suitable strain of organism (like in case of E. coli: DH5α for cloning and BL21-DE3 for expression); fermenters or bioreactors (preferably 1 litre); Spectrophotometer (for quantification) and for downstream processing: centrifuge (for cell harvesting); fractionators and chromatography systems (preferably column).

2.3 Hypothetical Production Methodology

A small new venture can be started by following the production flowchart (see Figure 1). Efficient coordination between steps will ensure steady production with reproducible yield.

![Figure 1: A Pilot-scale Production Flowchart for Filgrastim](image)

The concept of storage is of prime importance here. Though the temperature effect had been studied earlier (Tivnnann et al., 1996; Skrlin et al., 2010), a detailed temperature profile study investigating the effects of storage conditions on preservation of rhu-G-CSF is lacking. It has been reported that G-CSF can be stored at 4°C for up to two weeks without significant loss of biological activity (Tivnnann et al., 1996). A recent comparison between a biosimilar and reference filgrastim has revealed that both can be stored at 2-8°C for long and when kept under stress (at 40°C) for 12 weeks, the amount of filgrastim decreases within acceptable range (Skrlin et al., 2010).

3. MARKET OVERVIEW

3.1 Major Companies Producing Filgrastim (in Alphabetical Order)

Amgen, Inc. (USA); Amoytop Biotech (China); BioSource International; Calbiochem; Cell Sciences; Dr. Reddy’s...
Lab (India); M/S. F. Hoffmann La Roche Ltd. (Switzerland); Leinco technologies, Inc.; Peprotech; Prospec – Tany technogenes Ltd. (USA); Shantha Biotechniques (India).


3.2 World Market Scenario

By the end of 2004 the total world market value for Colony Stimulating Factors was estimated to be a staggering US $3.6 billion. From 2000 onwards, the market growth rate has been about 16%, which is highly impressive. It highlights the need for the recombinant human G-CSF.


With transnational sales of about US $1.8 billion, Neupogen (by Amgen, Inc.) has been rated as the one of the top pharmaceutical products (recorded in 2004). More than 20 additional companies with activities in the biogeneric field of G-CSF were identified in a search conducted by La Merie Business Intelligence. The results were published in the October 17 issue of R&D Pipeline News, edited by La Merie.


Biotechnology initiatives in India and China have lead to more than 20 domestic manufacturers of G-CSF in China and at least seven Indian companies with proprietary manufacturing. Due to extremely small domestic markets (e.g. Indian market size was only US $25 million due to low priced products), the Asian companies are collaborating with Western enterprises (GeneMedix, BioPartners and Dragon Pharmaceuticals).


This is reflected in the predicted growth & market size for Filgrastim in India (from 2005 to 2007).

(Courtesy: www.biospectrumindia.com/content/features/agri/default.asp):

<table>
<thead>
<tr>
<th>2005</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-generic Molecule</td>
<td>Market Size</td>
</tr>
<tr>
<td></td>
<td>INR (crores)</td>
</tr>
<tr>
<td>G-CSF (Filgrastim)</td>
<td>200</td>
</tr>
</tbody>
</table>

To do this effectively, we need a mock questionnaire to conduct a personal tête-à-tête with a firm owner who is deciding to produce or has been producing some variant of Filgrastim. Some common questions can be: (a) Why do/did you invest your money on producing Filgrastim? (b) What is/was the motivation behind the venture? (c) Why do you think that Filgrastim is the next big thing? (d) Is it a biogeneric or simply a chemical? (e) Is there any special procedure that you are following/have followed? (f) What is the expected annual production target for 2011-12? (g) Do you think that your target will be enough to make a significant contribution in fulfilling the market demand? (h) Who will be your / who are your major customer base? (i) At what rate the market for hematopoietic growth factors are growing? (j) Can you please share some marketing strategies for your product? Satisfactory answers to these questions will solve the initial hurdles.

3.3 Trend Projection Analysis: Prediction of Sales

Suppose we have the sales data available for X company from 1995 to 2004:

<table>
<thead>
<tr>
<th>Year</th>
<th>r-hu-G-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>937</td>
</tr>
<tr>
<td>1996</td>
<td>1017</td>
</tr>
<tr>
<td>1997</td>
<td>1057</td>
</tr>
<tr>
<td>1998</td>
<td>1118</td>
</tr>
<tr>
<td>1999</td>
<td>1258</td>
</tr>
<tr>
<td>2000</td>
<td>1225</td>
</tr>
<tr>
<td>2001</td>
<td>1347</td>
</tr>
<tr>
<td>2002</td>
<td>1845</td>
</tr>
<tr>
<td>2003</td>
<td>2523</td>
</tr>
<tr>
<td>2004</td>
<td>2916</td>
</tr>
</tbody>
</table>

All sales are in million US $. From this, we can predict the total sales for X for the next ten years. First let’s represent it graphically (see Figure 2). In the figure, year 1 represents 1995 and year 10 represents 2004. From the plot, it’s clear that the series of data follows a parabolic
trend ($R^2 = 0.95$). Applying the method of least squares, the equations are:

$$\Sigma y = na + b\Sigma x + c\Sigma x^2$$
$$\Sigma xy = a\Sigma x + b\Sigma x^2 + c\Sigma x^3$$
$$\Sigma x^2 y = a\Sigma x^2 + b\Sigma x^3 + c\Sigma x^4$$

By solving the above equations, the value of $a$, $b$ and $c$ are found to be 1255.8, -215.7 and 37.761 respectively. So the final equation becomes:

$$y = 1255.8 - 215.7x + 37.761x^2.$$ 

Now, putting $x$ to be 11, 12, 13, 14 and 15; we can make out the total sales projection for next 5 or 10 years: (this will reflect the target market value in the world).

### 4. COMPETITIVE ADVANTAGES

#### 4.1 How to Produce a Better Variety of r-hu-G-CSF than that Existing in Market?

We may have to consider and compare the existing varieties for this. A comparison for 3 different farms have been made (see Table 1). The principal factors that need to be considered are: (a) Quality: Using M13 mutagenesis and XbaI is a good idea since the amplified recombinant G-CSF analogs will show much more transformation efficiency than other variants. The use of cellulose columns will also increase the quality of the product. Purity & specificity of the drug will be much higher than other anti-cancer drugs. (b) Features: It must possess all the desired characteristics of normal G-CSFs & will act better than chimeric monoclonal antibodies. (c) Location: The plant has to be set up away from the main city to avoid any problem of waste disposal or pollution directly affecting the population. (d) Price: It has to be economic and effective at the same time.

### Table 1

Comparison of r-hu-G-CSF for Various Firms. Here ‘mcg’ refers to microgram. ‘IU’ Refers to International Unit. ‘kD’ refers to kilodaltons.

<table>
<thead>
<tr>
<th></th>
<th>Amgen, Inc. (USA)</th>
<th>Amoytop Biotech (China)</th>
<th>Prospec -Tany Technogenc Ltd.(USA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalogue No.</td>
<td>Not Available</td>
<td>SJA02</td>
<td>CYT-220</td>
</tr>
<tr>
<td>Source</td>
<td>$E. coli$ (strain FM5)</td>
<td>$E. coli$</td>
<td>$E. coli$</td>
</tr>
<tr>
<td>Molecular Mass</td>
<td>18.8 kD</td>
<td>18.788 kD</td>
<td>18.8 kD</td>
</tr>
<tr>
<td>Appearance</td>
<td>Solution</td>
<td>Solution</td>
<td>White lyophilized powder</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Purity</th>
<th>&gt; 99% (HPLC)</th>
<th>&gt; 97% (PLC)</th>
<th>&gt; 98% (HPLC, SDS-PAGE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endotoxin level</td>
<td>Not Available</td>
<td>&lt; 10 EU / 3.0 mg</td>
<td>&lt; 0.1 ng/ mcg of G-CSF</td>
</tr>
<tr>
<td>Activity</td>
<td>Not Available</td>
<td>0.02 - 0.06 ng/ml</td>
<td>10 µIU/mg</td>
</tr>
<tr>
<td>Formulation</td>
<td>300 mcg / 1ml vial</td>
<td>Lyophilized from a 0.2 µm filtered solution of 10 mM Acetate Buffer</td>
<td>Lyophilized after extensive dialysis against 10 mM Sodium Acetate buffer (pH = 4)</td>
</tr>
<tr>
<td>Storage</td>
<td>-20 to -80°C</td>
<td>-20 to -70°C</td>
<td>-20 to -70°C</td>
</tr>
</tbody>
</table>

### 4.2 SWOT Analysis

#### 4.2.1 Potential Strength

1) Strength lies in providing the best quality product to the customer.
2) There has to be 24x7 work culture. Production can’t stop.
3) Provision should be made for insurance premium & incentives for the employees.
4) Location will be away from main city. It will not affect the population.
5) Excellent R&D facilities with Top class scientists.
6) Ability to achieve break-even in the starting year only.

#### 4.2.2 Potential Weakness

1) Inexperience regarding starting a new business.
2) Very difficult to optimize the production.
3) Chances of contamination will be very high for G-CSF production.

#### 4.2.3 Potential Opportunities

1) Market is growing at a very impressive rate.
2) If neutropenia can be cured by the product, cancer patients will have a ray of hope.
3) Can out market the production of conventional chimeric Monoclonal Antibodies.

#### 4.2.4 Potential Threat

Threat may come from the already existing health clubs for an alternative approach to treat neutropenia. But the overall pattern, approach and devotion to the goal will help overcome any such potential threat.

### 4.3 PEST Analysis

#### 4.3.1 Political Analysis

From political side, the stage is set to start G-CSF
production since the Govt. encourages such entrepreneurial venture as it also creates job opportunities. Therefore there should not be any significant political problem in any new venture.

4.3.2 Economic Analysis

One may start the venture with US $ 60,000. Out of this one’s own share capital may be about US $ 20,000. The remaining one may take as a secured loan from a leading commercial bank. Also, it has to be shown that the breakeven will be obtained in the first year itself. So one may be able to make profit straightaway (occurrence of any unnatural complications will be purely unfortunate).

4.3.3 Social Analysis

This area needs to be monitored carefully. Not much problem is anticipated in a developed country. But in a developing country, majority of the people is under the poverty line. So, we’ve to keep in mind the economic status before deciding the final cost as poor people could not afford highly priced drugs. However, it is evident that with increase in production of variants of Filgrastim, the cost will eventually come down.

4.3.4 Technological Analysis

Any developed country has proper technology to produce the desired product. Let’s review the condition in a developing country like India. The Genetic Engineering Approval Committee (GEAC) and Review Committee on Genetic Manipulation (RCGM) have already approved the use of E.coli for carrying out recombinant experiments. They are non pathogenic & are use for a host of genetic engineering experiments. Recently Dr. Reddy’s Lab has been granted approval for r-hu-G-CSF production. The latest fermentation technology for culturing the recombinant E.coli cells should also be encouraged. To observe the 3D structure, latest molecular viewing softwares can be utilized. I’ll take help of latest molecular viewing softwares. Highly efficient and sophisticated techniques like DEAE cellulose columns will be used for chromatography. For subsequent analysis, HPLC will be used. Sincere attempts should be made to produce the product under strict monitoring & control. One also needs to adhere to the rules issued by various Regulatory & Competent authorities.

5. FINANCIAL OR ECONOMIC FORECASTS

5.1 Proforma Income Statement (from 2007-11)

In preparing a hypothetical income statement, we assume that the price per unit for a single 1 ml vial (containing 300 microgram of the desired product) is about US $ 300. Also we are assuming that the start-up company may sale about 0.01 % of that of any international pharma giants (or MNCs). So for a whole year, say 2007, the total number of vials sold by the firm will be around 1150. So, the total sales for 2007 will be around US $ 3,45,000. For subsequent years, it is assumed that the sales will grow by 16% (16% growth rate is overall prediction for market

<table>
<thead>
<tr>
<th>YEAR</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of vials sold</td>
<td>1150</td>
<td>1334</td>
<td>1547.44</td>
<td>1795.0304</td>
<td>2082.235264</td>
</tr>
<tr>
<td>Total Revenue</td>
<td>15525000</td>
<td>18009000</td>
<td>20890440</td>
<td>24232910.4</td>
<td>28110176.06</td>
</tr>
<tr>
<td>Rent</td>
<td>240000</td>
<td>240000</td>
<td>240000</td>
<td>240000</td>
<td>240000</td>
</tr>
<tr>
<td>Salaries &amp; wages</td>
<td>450000</td>
<td>450000</td>
<td>450000</td>
<td>450000</td>
<td>450000</td>
</tr>
<tr>
<td>Electricity Charges</td>
<td>6000</td>
<td>10000</td>
<td>14000</td>
<td>20000</td>
<td>25000</td>
</tr>
<tr>
<td>Raw materials</td>
<td>1500000</td>
<td>1700000</td>
<td>2050000</td>
<td>2200000</td>
<td>2400000</td>
</tr>
<tr>
<td>Stationary</td>
<td>500000</td>
<td>1000000</td>
<td>1500000</td>
<td>2000000</td>
<td>2500000</td>
</tr>
<tr>
<td>Depreciations</td>
<td>120000</td>
<td>120000</td>
<td>120000</td>
<td>120000</td>
<td>120000</td>
</tr>
<tr>
<td>Fermenter maintenance</td>
<td>400000</td>
<td>400000</td>
<td>400000</td>
<td>400000</td>
<td>400000</td>
</tr>
<tr>
<td>Advertisement/Marketing</td>
<td>300000</td>
<td>300000</td>
<td>250000</td>
<td>200000</td>
<td>150000</td>
</tr>
<tr>
<td>Downstream processing</td>
<td>700000</td>
<td>780000</td>
<td>856000</td>
<td>880000</td>
<td>900000</td>
</tr>
<tr>
<td>Insurance Premium</td>
<td>60000</td>
<td>60000</td>
<td>60000</td>
<td>60000</td>
<td>60000</td>
</tr>
<tr>
<td>Other costs</td>
<td>500000</td>
<td>550000</td>
<td>600000</td>
<td>650000</td>
<td>700000</td>
</tr>
<tr>
<td>Total Expenses/ Cost</td>
<td>4776000</td>
<td>5610000</td>
<td>6540000</td>
<td>7220000</td>
<td>7945000</td>
</tr>
<tr>
<td>Profit Before Tax (PBT)</td>
<td>10749000</td>
<td>12399000</td>
<td>14350440</td>
<td>17012910.4</td>
<td>20165176.06</td>
</tr>
<tr>
<td>Tax (32%)</td>
<td>3439680</td>
<td>3967680</td>
<td>4592140.8</td>
<td>5444131.328</td>
<td>6452856.34</td>
</tr>
<tr>
<td>Profit After Tax (PAT)</td>
<td>7309320</td>
<td>8431320</td>
<td>9758299.2</td>
<td>11568779.07</td>
<td>13712319.72</td>
</tr>
</tbody>
</table>
for G-CSF). The Income statement (hypothetical) is shown in Table 2. For the suitability of a developing country production, the figures are projected in INR.

5.2 Breakeven Analysis

The breakeven point \( Q \) is given by:

\[
Q = \frac{\text{Total Fixed Cost}}{(\text{Price per vial} - \text{Variable Cost per vial})}
\]

From the 1st year Profit & Loss Account: Total Fixed Cost is around US $28,222. Price per Vial is US $300 and Variable Cost per Vial is US $68. So, \( Q \) is 121,515 vials. So it’s possible to make profit in the first year itself, as shown by the projected income statement.

6. CONCLUSION / DECISION ON THE BUSINESS VENTURE

The market for Filgrastim in both developing and developed countries is expanding. The techno economic analysis reveals a significantly possible pilot scale production of recombinant human analogs of G-CSFs for their medical benefits. The SWOT and PEST analyses show that the business venture is possible, albeit with less difficulties in a developed country and with few concerns in a developing country. But the overall picture is in favor of the venture, provided we adhere to all the rules and regulations of the governing bodies and fully exploit the opportunities.

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References


