Entrepreneurship Proposal:
A gene therapy strategy to target hepatocellular carcinoma
based on conditional RNA interference

CPU_CHINA
Human Practice
2018
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1. Summary

Hepatocellular carcinoma (HCC) is a kind of primary liver cancer with a high mortality rate. Currently, there are various clinical treatment methods, including surgical resection, liver transplantation, local ablation, radiotherapy and chemotherapy. However, all kinds of therapies have corresponding side effects and patients' prognosis is not ideal.

In our project, under the control of small molecule tetracycline, through the "AND" gate composed of hTERT cancer specific promoter AND HULC liver cancer specific promoter, microRNA mediates silencing the mRNA of MAP4K4, the high expression protein of cancer cells, so as to achieve the controllable killing effect on liver cancer cells to reach the therapeutic purpose.

Involving in liver cancer incidence, clinical treatment status, project advantages, production process and cost accounting and some other aspects, our proposal aims to explore the possible commercial operation mode of the product preliminarily. As a careful and creative approach, We do business simulations of the project to consider how our project affects society and how market influences the direction of our project, and to integrate those considerations into the purpose, design, and/or execution of our project.

2. Background

2.1 Introduction of liver cancer

Liver cancer is predicted to be the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide in 2018, with about 841,000 new cases and 782,000 deaths annually. Rates of both incidence and mortality are 2 to 3 times higher among men in most world regions; thus liver cancer ranks fifth in terms of global cases and second in terms of deaths for males[1].

Primary liver cancer includes hepatocellular carcinoma (HCC) (comprising 75%-85% of cases) and intrahepatic cholangiocarcinoma (comprising 10%-15% of cases) as well as other rare types.
Its incidence is obviously regional, with the highest incidence in east Asia (about 50% in China) and sub-saharan Africa, accounting for about 85% of the total incidence[2]. Therefore, the research and development of related therapies/drugs is particularly important.

![Figure 1](image-url)  
**Figure 1:** The 15 most common cancers world (W) in 2018 are shown in descending order of the overall age-standardized rate for man. Source: GLOBOCAN 2018.

### 2.2 Diagnosis and treatment of liver cancer

#### (1) Diagnosis

Liver cancer has no obvious symptoms in the early stage, and patients are usually in the middle and late stage once they have obvious symptoms[3]. According to the current treatment level, the five-year survival rate of early treatment is more than 50%, while that of late treatment is less than 5%.

#### (2) Treatment

Different treatment options are selected according to the cancer process: surgical resection is preferred for early liver cancer. In the middle stage of liver cancer, the patients were treated with combination therapy to control the growth and metastasis of tumor cells. Patients with late stage liver cancer must be treated with systemic drugs to prolong their survival, using largely dividend
excision or interventional therapy combined with sorafenib.

Hepatectomy, liver transplantation, local ablation, embolization, radiotherapy and chemotherapy are commonly used for the treatment of liver cancer[4][5][6]. The advantages and disadvantages of various treatment methods is as follows:

Table 1. Comparison of existing therapies/drugs for liver cancer

<table>
<thead>
<tr>
<th>Therapies/Drugs</th>
<th>Indications</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial hepatectomy</td>
<td>Early liver cancer, the tumor has not grown blood vessels, and the patient’s liver function is good enough</td>
<td>The lesions can be removed at the maximum extent</td>
<td>Surgical complications such as bleeding and infection may occur, and the recurrence and metastasis rate of tumor is as high as 40% to 70% within 5 years after surgery</td>
</tr>
<tr>
<td>Liver transplantation</td>
<td>A small tumor patient with an unresectable tumor that has not yet invaded a nearby blood vessel</td>
<td>Can significantly reduce the risk of liver cancer again</td>
<td>Complications such as bleeding and infection may occur, and donor shortage is a major problem for this treatment</td>
</tr>
<tr>
<td>Liver ablation</td>
<td>small tumor patients</td>
<td>Economical, convenient, minimally invasive and less likely to cure cancer than surgery, but very helpful for some patients with fewer serious complications</td>
<td>Normal tissue around tumors is often damaged, so is not suitable for patients with tumors near major blood vessels, membranes, or major bile ducts</td>
</tr>
<tr>
<td>embolotherapy</td>
<td>A patient whose tumor is too large to undergo ablative treatment</td>
<td>Simple, safe, minimally invasive, and good efficacy</td>
<td>It reduces the blood supply to some normal liver tissue, impairs liver function and is not suitable for patients whose liver has been damaged by diseases such as hepatitis or cirrhosis</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>A common treatment for patients who are</td>
<td>An important method in</td>
<td>It will damage normal liver cells, often with</td>
</tr>
<tr>
<td>Systemic treatment</td>
<td>unable to undergo surgery in late stages</td>
<td>comprehensive treatment, some can achieve precision treatment</td>
<td>side effects including skin damage, nausea and vomiting, and blood cell decrease</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------------------------</td>
<td>-------------------------------------------------------------</td>
<td>---------------------------------------------------------------------</td>
</tr>
<tr>
<td>Systemic chemotherapy: effective drugs include doxorubicin, 5-fluorouracil and cisplatin</td>
<td>FOLFOX4 regimen systemic chemotherapy for &quot;locally advanced and metastatic liver cancer not suitable for surgical resection or local treatment&quot;</td>
<td>Appropriate systemic chemotherapy can prolong the progression-free survival, improve remission and disease control</td>
<td>Only a small number of tumors can be shrunk. Severe side effects such as hair loss, nausea and vomiting, diarrhea, and blood cell loss often occur</td>
</tr>
<tr>
<td>Molecular targeted drug: sorafenib</td>
<td>Standard treatment for advanced patients</td>
<td>The target ability is good, which can shorten the course of treatment, reduce the rate of administration, have significant efficacy and less toxic and side effects</td>
<td>Common adverse reactions are diarrhea, weight loss, hand-foot syndrome, skin rash, myocardial ischemia and hypertension</td>
</tr>
<tr>
<td>Immunotherapy: Nivolumab(Opdivo)</td>
<td>indicated for the treatment of patients with HCC who have been previously treated with sorafenib</td>
<td>High efficiency; Low toxic and side effects; Helps restore the body's immune system</td>
<td>High price, and may appear fever, red rash and other allergic reactions</td>
</tr>
</tbody>
</table>

In fact, as mentioned above, most patients are at the late stage when diagnose. The main treatment of late state cancer is chemotherapy or targeted drugs[7]. Even if the patient is lucky enough to receive surgical treatment, the postoperative recurrence rate is reaching 60-70% and the five-year tumor-free survival rate is only 26%. After surgical treatment, patients still need to take medicine or other treatment to improve the survival rate. Now there is a shortage of drugs/therapies with good efficacy and few side effects. And develop a safe and effective treatment for liver cancer is still a difficult problem to be solved.

2.3 Gene therapy and RNA interference
In 1989, the National Institute of Health conducted the world's first successful gene therapy study[7]. Studies related to gene therapy have been developing rapidly since then. Up to 2017, 1,476 gene therapy schemes/drugs have entered phase I clinical trials around the world[8]. Currently, 6 drugs have been approved and marketed. The pharmacodynamic molecules of gene therapy also involve tumor suppressors, transcription factors, oligonucleotide, siRNA and some other types [8]. Gene therapy, as a novel therapy, has attracted more and more attention.

In 2006, Andrew Z. Fire and Craig C. Mello won the Nobel Prize for physiology or medicine because their outstanding contributions in the field of the discovery of RNA interference-gene silencing by double-stranded RNA[9]. At present, the technology is developing and applying in many fields such as drug development, clinical treatment and disease diagnosis. The most common way to use it in clinical treatment is to develop new gene drugs that inactivate disease-causing genes. On August 10th, 2018, FDA approved Alnylam's siRNA drug Onpattro (patisiran) injection for an adult peripheral nerve disease (polyneuropathy) caused by hereditary transthyretin-mediated amyloidosis (hATTR).

MiRNA is an important type of molecular that plays the role of RNA interference. However, some adverse reactions may occur when such tools directly and continuously knock out the target gene, so it is necessary to regulate MiRNA[10].

3. Market Analysis

3.1 Market of drugs for liver cancer

According to the Research of Grand View Research, the global liver cancer drug market was estimated to be about $424 million in 2016, and increased with a compound annual growth rate of 19.4%. It is estimated to be about $1.47 billion by 2022[11]. Drug development market of liver cancer is vast.
The total size of China’s liver disease drug market has increased from 233.28 billion yuan in 2010 to 537.05 billion yuan in 2015. The total size of China’s liver disease drug market in 2014 and 2015 was 459.61 billion yuan and 537.05 billion yuan respectively, with an increase rate of 16.85%. It is predicted that the total market size of hepatopathy drugs will maintain an annual growth rate of 15-20%[12]. Liver cancer mainly occurs on the basis of chronic liver disease or cirrhosis (accounting for 70% to 90% of all liver cancer)[12]. Therefore, the size of liver cancer drug market cannot be underestimated.

3.2 Current characteristics of drugs for liver cancer

(1) The treatment of liver cancer is reliant on drugs because of the late diagnosis.

As mentioned above, only 10-30% of the patients were treated surgical resection, and the recurrence rate in 5 years after surgery was still more than 70%[13]. Also, treatment for surgical prognosis is in great demand for drugs. Other patients who cannot be treated surgically need chemotherapy or targeted drug therapy. Treatment for late stage liver cancer is particularly dependent on systemic drugs[14].

(2) Lack of effective treatment seriously

Drugs for liver cancer play a key role in maintaining the patients’ life quality. At present, liver cancer drugs are all chemical drugs with poor targeting and high
side effects, which is related to the insensitivity of liver cancer to chemotherapy drugs and the possible presence of primary resistance genes. Clinical results showed that the overall chemotherapy effect of liver cancer was not good[15]. Meanwhile, most of patients suffered from liver function damage, which lead to drug metabolism impair; and the adverse reactions of chemotherapy were very severe. Therefore, liver cancer patients need effective targeted drugs urgently.

Currently, sorafenib is the only targeted drug that is applicable to advanced liver cancer in China.

3.3 Target groups

Both the NCCN clinical guidelines and the Chinese liver cancer treatment guidelines stipulate that only systemic treatment is practicable for patients with late stage liver cancer who cannot be surgically removed. These patients account for about 2/3 to 1/2 of the total number of liver cancer patients, which means the target groups of targeted drugs for liver cancer is about 900,000 people worldwide and 500,000 among them are in China[16]. The target groups of the product involved in our iGEM project are these patients whose liver cancer is in middle or late stage.

4. Competition Analysis

4.1 Competition with other liver cancer drugs

(1) Chemotherapeutic agent for liver cance

Commonly used chemotherapy drugs for liver cancer include anthracycline, fluorouracil, platinum, camptothecin and gemcitabine. Chemotherapy is a common treatment for liver cancer, but one of the major drawbacks is that the side effects are seriously, causing pain to the patients. And once severe side effects occur, the treatment process will be affected. In terms of curative effect, hepatic artery chemoembolization is the main method for the treatment of advanced liver cancer. But kang et al. showed that the total effective rate was only 62% at 1 month after TACE treatment alone, and the survival rate at 24 months significantly decreased from 92% to 40%[17].
(2) Targeted drugs for liver cancer

① the only targeted drug for liver cancer in China——sorafenib

Sorafenib is a liver cancer treatment drug developed by Bayer. It is the only targeted drug approved to treat liver cancer in China. Sorafenib is an oral multi-kinase inhibitor that affects both the tumor cell signaling system and the tumor vascular system. In terms of clinical efficacy, sorafenib is the only drug proved so far that significantly extend the total survival of patients with advanced liver cancer. Its emergence has indeed ushered in a new era of targeted therapies for liver cancer and brought hope for systemic treatment of advanced liver cancer. Sorafenib is expected to remain one of the few oral targeted liver cancer drugs for a long time to come.

Figure 5. National sales of sorafenib from 2005 to 2012, IMS drug data. 2013

A. Sales

Sorafenib’s sales have risen since its launch in 2005. According to IMS, global sales of the drug rose from $45.86 million in 2006 to $605 million in 2012 (Figure 5) and could peak at $0.8 billion to $1 billion in 2017[18].

B. Weakness

a. Prices of soraffinib are too high and not covered by medical insurance. At present, there are imitations in India and other places on the market. Although the price is only 10% of the original research drug, the quality and safety of them are not guaranteed. Patients take these imitations with huge risks.
b. Sorafenib has severe hand, foot and skin side effects and the tumor will develop drug resistance at an average of 17.6 weeks (about 4 months)[18]. Whether the drug can achieve the desired effect is required for patients with hepatitis. Data showed that the patients who were combined with Hcc and hepatitis b cirrhosis were less likely to benefit from sorafenib than those who were combined with Hepatitis c cirrhosis.

② Several pending or unapproved drugs, such as lenfatinib and regafinib, will expose project products to greater competition while bring more choices to patients. However, according to the current research, these drugs do not occupy more advantages, and they cannot replace sorafenib in the short term as the choice of first-line treatment.

(3) Immune therapy

At present, car-t therapy is showing a continuous high trend, and the expectation of patients is higher. However, there is still a technical block in the treatment of solid tumors, and there is no complete car-t therapy in the field of liver cancer for clinical application. Anyway, CAR-T therapy is a potential rival.

(4) Interferon therapy

Interferon therapy is used as an adjuvant therapy for postoperative recovery. Recombinant interferon can not only reduce the inflammation caused by liver cancer, but also reduce the chance of liver cancer. Recombinant interferon (RiFN) is the only proved effective adjuvant therapy.

4.2 Competition with RNAi class drug

(1) Competition with different types of RNAi drugs

On Aug 10, 2018, FDA approves Alnylam’s siRNA drug Onpattro (patisiran) injection for the treatment of adults peripheral nerve disease (polyneuropathy) caused by hereditary transthyretin-induced amyloidosis (hATTR)[19]. At present, siRNA drug is the most mainstream type of RNAi drug, with abundant studies and few studies on miRNA. However, with the further study, some experimental results showed that miRNA have lower cytotoxicity and higher efficiency than siRNA[20].
(2) **Competition with RNAi technology**

① Biotech companies are increasingly competing in RNAi technology and starting to invest in research and development.

Germany’s Ribyoy harma was the first company to obtain the siRNA silence gene patent. Sima and Alnylam have important patents to keep RNA molecules stable, and some pharmaceutical giants such as Abbott Laboratories of the United States and biotech giants such as Genentech have also started to work on RNAi.

② Major drug makers are scrambling for patents related to therapeutic RNAi technology in order to take the market to its highest point as soon as possible. Alnylam, for example, has licensed patents to 14 companies, including Dharmacon, sigma-aldrich and Invitrogen, since it was founded in 2002, with its technological lead in the field of RNAi therapy.

### 5. SWOT Analysis

5.1 **S(strengths)**

(1) The drug action has a high degree of cancer tissue specificity, which can greatly avoid the damage to healthy tissue and reduce the organ damage and patient pain caused by side effects of the drug.

(2) Pri-mirna was used to express prodrug in an innovative manner. Compared with other RNAi drugs, it has low cytotoxicity and higher biological activity. The effect on other endogenous mirnas was minimal compared to the currently more prevalent siRNA/shRNA types.

(3) With high controllability of drug use, the number of effector miRNA can be regulated by small molecule compound doxycycline, which is conducive to further control of drug action and toxic and side reactions in terms of time and space. The product can be used for individualized treatment of liver cancer and combined drug use through rational study of the mathematical relationship between doxycycline and curative effect and control of doxycycline dosage.
(4) MAP4K4, the target selected by this drug, has a great prospect. MAP4K4 is involved in a wide range of physiological processes involving systemic inflammation, metabolic disorders, cardiovascular disease and cancer[21]. This molecule is highly expressed in a variety of tumor cells and theoretically can achieve good therapeutic effect by drug targeting[22]. In the case of HCC, MAP4K4 overexpression, especially as an independent predictor of poor prognosis in HCC patients, is of great therapeutic significance to inhibit its expression[23].

5.2 W(weaknesses)

(1) The route of drug administration is mainly injection, accompanied by pain, and patients may prefer to choose oral drugs.

Gene drugs are high-tech products with few markets, and RNAi drugs are new research drugs. The public has no understanding of their scientific nature, reliability and rationality, and may hold a wait-and-see attitude or distrust. Because RNAi is a new technology, the cost of production has not improved, so the price will be slightly higher than traditional drugs.

5.3 O(opportunities)

(1) Drug market opportunities for liver cancer: in recent years, the prevalence and mortality of liver cancer rank among the top of various cancers. In addition, affected by people's living habits such as diet, the incidence of alcohol liver, liver cirrhosis and other liver diseases is also high. Without timely and effective treatment, these "small liver diseases" are at great risk of turning into liver cancer. At present, the advanced treatment research is mostly seen in non-solid tumors, and the development of drugs for liver cancer by biological companies has little attention.

(2) Most traditional chemical drugs are not effective and expensive, having large side effects, relatively good curative effects, and cannot meet the requirements of patients. Many researchers have turned their attention to gene therapy. At present, the development of gene drugs is in a good situation, as can be seen from the popular car-t therapy. However, gene drugs have a large gap in the
market of liver cancer drugs, which is a great opportunity for the product.

(3) Rapid advances in RNAi technology: by manipulating the cell’s RNA (genetic messenger), RNAi interferes or suppresses target genes to inhibit the formation of proteins that cause disease. The scientific community generally believes that RNAi technology is highly targeted and could potentially yield promising therapeutic drugs for refractory diseases including cancer, blindness and AIDS. As an emerging research direction, RNAi currently has no drug approval in the field of liver cancer.

(4) In recent years, China has been increasing its support for the biomedical industry. In the "Twelfth-Five" national Strategic emerging Industry Development plan issued by the state council, the biological industry was identified as one of the seven strategic emerging industries, and its strategic position in the national economy was determined. In the 13th five-year Biological industry development plan for the development of the biological industry, it is proposed to realize the original innovation of drugs in the field of tumor and develop biological treatment products such as RNA interference drugs. In fiscal year 2015/2016, China’s investment in research and development in the field of biopharmaceuticals increased by 27.5 percent, and the investment in related fields will exceed 300 billion us dollars in the past three years[24].

5.4 T(Threats)

Although the research progress of RNAi technology has made great progress, the new drug developed with RNAi technology is faced with the practical problems of drug instability, difficulty in quantification and accurate delivery of drugs to target tissue, etc., various biotechnology companies have made different attempts in this regard, and used virus vectors and chemical methods for regulation. The vector virus has the risk of causing immune response, but these risks are decreasing with the continuous improvement of design. (Refer risk control section)
6. Business model

6.1 Project Summary

2018 iGEM CPU_CHINA team choose RNAi as skeleton, through the design control and cross-border use RNA polymerase chain pri - mature miRNA, and the disease specific promoter "AND" targeted cancer of the liver tissue, again through the small molecular substances control the amount of mature miRNA genes can significantly reduce drug adverse reactions such as damage in normal cells, compared with the traditional method of treatment of liver cancer or drugs has a great advantage.

6.2 Time Nodes

Under the premise of quality assurance, the development speed should be accelerated as far as possible in view of the fact that many new drugs for liver cancer are on the market and the formation of competitive situation.

<table>
<thead>
<tr>
<th>Plan</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete the whole design, complete the theoretical experiment and verify the effect</td>
<td>Seven months</td>
</tr>
<tr>
<td>Site construction, personnel recruitment, equipment and supplies procurement</td>
<td>A year and a half</td>
</tr>
<tr>
<td>Industrial construction and in vitro validation</td>
<td>Two months</td>
</tr>
<tr>
<td>Perfect GMP workshop production specification and related information</td>
<td></td>
</tr>
<tr>
<td>Industrial efficacy evaluation and pharmacokinetic test, pilot test and other improvements</td>
<td>Two months</td>
</tr>
<tr>
<td>In vivo evaluation of pre-clinical animal model, process optimization</td>
<td>One month</td>
</tr>
<tr>
<td>The data of pre-clinical safety evaluation is perfect</td>
<td>Two months</td>
</tr>
<tr>
<td>Collect, sort and declare the data of clinical application</td>
<td>One month</td>
</tr>
<tr>
<td>Optimization and perfection, related patent application</td>
<td>Four months</td>
</tr>
</tbody>
</table>

6.3 Business model

(1) Focus on research and development in the early stage and make perfect patent layout

(2) After the establishment of an effective patent protection system and products,
industrial authorization and intellectual property transfer shall be carried out

(3) In the later stage, the industrialization of new pri-mirna gene vectors or RNAi drugs for other genetic diseases was conducted, and the national market layout and international cooperation layout were conducted in the multi-regional preparation center.

(4) The acquisition and merger of pharmaceutical giants are not excluded

(5) The company went public in six years.

7. **Productive technology and quality criterion**

7.1 **Production process**

- Cloning the interest into a viral vector
- Transformation
- Culture E.coli
- Viral plasmid extraction
- Virus Packaging and detection

7.2 **Quality Control**

According to "the Guidance for the Human Somatic Cell Therapy and Gene Therapy Issued by FDA in 1998, should determine the characteristics of Cell identity, potency, viability, purity, Adventitious agents (such as bacteria, fungi, mycoplasma, etc.) and General Safety Test[25]."

On the purity, identity, potency and Adventitious agents of the selected carrier, including Sterility, Mycoplasma Adventitious viruses inspection. For adenovirus vector, should detect particles vs. infectious units, virus drops, adenovirus, adeno-associated virus replication type.

For aseptic drug products, Sterility, identity, potency, endotoxin and general safety should be considered.

As our product is expected to be prepared in the form of injection, it should
meet the quality requirements for injection in the Chinese pharmacopoeia in the corresponding years.

8. Risk control

8.1 Production risk

(1) **Resource supply:** the production of drugs in the design mainly involves raw materials such as E. coli, target genes and required reagents. Before the formal production, at least one alternative partner should be identified, and another raw material supplier should also be contacted if there is any problem with the raw material.

(2) **Process equipment:** production equipment and process technology should be synchronized with new drug innovation technology. Timely maintenance of instruments and equipment, timely repair if problems occur.

(3) **Operators:** the technical level and proficiency of the production operators shall meet the corresponding requirements of GMP.

The most important aspect of drug production is quality. Therefore, strict requirements should be imposed on the workers, checks on the process layer by layer and strictly checks whether the finished products meet the quality standards. In case of production failure, the bidder shall be severely punished.

8.2 Risks of technology

(1) The technical problems appeared in the drug development process bring risks to the production process, and excellent technicians are the only way to solve these technical problems.

(2) **Life of technology:** For example, due to the rapid pace of technology update, the new technology research and development application will soon be replaced by the updated technology, resulting in a short technology life cycle and the initial investment funds not be recovered. We should pay close attention to the development trend of the production industry.

8.3 Security risks
Strictly check every aspect in the process of production and check the production safety of the production workshop regularly. Once problems such as bacterial contamination and bacterial liquid leakage occur, the production in the contaminated area should be stopped immediately and cleaned up.

8.4 Financial risk
(1) Select reliable staff to strictly manage the company's finances: timely liquidate the company's profit and loss, and ensure that there is sufficient liquidity for the daily operation of the company and the needs of the company.
(2) According to the different development stages and the characteristics of funds required for new drug projects, captain should be timely and correctly financed in a timely manner at important moments such as effectiveness verification, industrialization construction and pre-clinical evaluation to ensure the liquidity of funds and avoid the financial risks improper financing brought. If the funds are scarce, considering the problems of high risk of anti-hepatocarcinoma drug development and long development cycle, joint development of multiple enterprises can be chosen.
(3) Transnational development may have exchange rate risk.

8.5 Management risk
Management of talent and organizational structure are the main factors affecting management risk. Persons with high ability and personal quality should be selected as managers to plan a reasonable organizational system, so as to maintain the operational sensitivity and information dissemination in the production and marketing stages, and improve the coordination of the organization system.

8.6 Market risk
(1) As a new treatment, gene therapy, has played an important role in the medical field in recent years. Even so, the related technology is still not mature, and the virus vector still has security problems. Adverse reactions are the key factors that restrict the stable presence of drugs in the market. Therefore, we should always insist on innovation and constantly improve our technology and products.
same time, the quality of drugs should be strictly controlled and the long-term status of products in the market should be stabilized.

(2) Gene drugs require high R&D costs. From this perspective, this product may not have an advantage over chemotherapy-targeted drugs led by sorafenib. Therefore, as a latecomer, we should not be overly demanding and profitable on the premise of high costs. Instead, we should adopt a strategy of winning sales to occupy the market.

(3) The country has strict management over the listing of drugs, and there are many related laws and regulations. Unreasonable marketing channels and marketing methods may cause risks. Therefore, it should be legally advertised.

In short, we should do more market research, clarify market trends and strengths(or weaknesses) of the product, grasp market opportunities, and make the most use of its advantages. Meanwhile, we should clarify its disadvantages and improve as much as possible in these areas.

8.7 Competitive risk

Research on gene therapy has been very popular in recent years. It is not excluded that competitors have proposed similar projects. Therefore, the pace of industrialization should be accelerated so that we could win the opportunities. After acquiring the core patent for conditional RNAi, the team should begin to develop new targets and new diseases to expand the use of the therapy. In this way, even if there appears similar therapies, we can be familiar with the market trend and stay in the dominant position in the competition. In addition, competition for other liver cancer drugs may also have a certain impact on our products. Therefore, under the premise of ensuring the advantages of our products (good targeting, high controllability, and low side effects), we should lower the selling price as much as possible, thus occupying a market position in the competition for similar drugs.
9. Analysis of cost and price

Table 3. The price of the raw materials and instruments

<table>
<thead>
<tr>
<th>Content</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-dried plasmid</td>
<td>¥6,000/4μg</td>
</tr>
<tr>
<td>pAAV-Helper</td>
<td>¥1,000/1μg</td>
</tr>
<tr>
<td>E.coli</td>
<td>¥290/20 × 100μl</td>
</tr>
<tr>
<td>HEK 293 T cells</td>
<td>¥5,091/100ml</td>
</tr>
<tr>
<td>GE Xcellerex® XDR-50 MO Microbial Fermentor</td>
<td>$300,000</td>
</tr>
<tr>
<td>AKTA Flux Tangential Flow Filtration System</td>
<td>$80,000</td>
</tr>
<tr>
<td>AKTA pure 150 Chromatography System</td>
<td>$80,000</td>
</tr>
<tr>
<td>GE WAVE 25 Bioreactor System</td>
<td>$80,000-100,000</td>
</tr>
<tr>
<td>GE AKTA ready flux single-use system</td>
<td>$200,000</td>
</tr>
</tbody>
</table>

9.1 Cost of drug production

Because the drugs based on the new RNAi technology are rare, it is difficult to find a reference, and the cost estimation is limited by conditions. This project does not involve specific industrial development costs so we only make simple estimates related with genetic drugs. In theory, pri-mi RNA has higher synthesis cost than RNAi oligo-chain molecules such as siRNA and shRNA due to its long length and current process requirements, but it can eliminate required fee in the steps of immune cell separation compared with the current popular CAR-T therapy. According to the consultation of related drug companies, the current CAR-T project has a treatment cost of about 50,000 yuan for one person and one course including preparation, consumables, transportation, storage and other processes. The number of treatment stages of the patients determined by their own recovery. Due to the use of newer technical methods, the cost of the project can be higher than this level with[1] in a reasonable range.

9.2 Analysis of the price

(1) Analyze the technology and the competition in the market, respectively, based on the price of existing drugs. A simple estimate of the price or cost of the project
has been done based on the only siRNA drug Onpattro listed on Alnylam, represented as RNAi drugs. Use the cost-effectiveness analysis of sorafenib on behalf of liver cancer drugs as a reference, we assume that our product has the same effects as sorafenib, so a more reasonable price range is should be made to win market competition.

Onpattro was approved for listing in the United States on August 10, 2018. It is used to treat nerve damage caused by hereditary transthyretin amyloidosis (hATTR) and is the first drug approved for the treatment of this indication. hATTR is a hereditary disease and is a serious and fatal rare disease. The patient’s life expectancy is only 2-15 years from the onset of symptoms. Onpattro, similar to other top-notch rare-drug drugs, is expensive and priced at $450,000, or about $345,000. If the influence of other factors on pricing is excluded, it is assumed that the direct or indirect cost of the drug technology cost and transportation cost are the same as our project, and the difference between the company strategy and the pricing strategy is excluded, we only take the influence of the target customer quantity on pricing into consideration. The global hatter patients are about 50,000 patients and the audience of our project is about 500,000 people, which is roughly converted. The price of this project is basically 3.5-45 million US dollars which is reasonable. It can also be inferred that the cost of drug development using RNAi technology should be lower than this number.

There have been many studies on the cost-benefit analysis of Solafenib, mostly using Markov model and comparing it with other therapies, and the results are basically similar. According to the study of chen et al. about Sorafenib versus Transarterial chemoembolization for advanced-stage hepatocellular carcinoma: a cost-effectiveness analysis[26], Monthly costs were estimated with the frequency and unit cost of drugs and procedures (that include treatments for HCC, cirrhosis and adverse events derived from associated drugs and procedures), inpatient and outpatient visits, laboratory testing and imaging examination, and all were converted to U.S. dollars in 2016.
The cost of TACE, including hospitalization, specialist treatment and various examinations during hospitalization in China ($3,170) is lower than the monthly cost of dose-adjusted sorafenib ($4,060). For the effects of two therapies are similar, TACE is more effective. If we assume that our effect in advanced liver cancer is the same as the former two, and the cost of using our therapy treatment is less than $3,170 per month, then it can be considered as extremely competitive; if it was less than $4060 per month, then it can be considered as having the equal competitiveness to sorafenib.

However, if full-dose sorafenib is used, the cost will increase greatly as well with the increase in curative effect. According to the Markov model, the per capita QALY of the full-dose sorafenib is 0.060, and the per capita cost is $6061.73. The benefit is higher than TACE as the optimal strategy for the treatment of advanced liver cancer. If the product can achieve the effects in the level of a full-dose sorafenib treatment, the price and associated costs need to be less than $60,061 per month for maximum benefit.

(2) Formulate pricing strategies based on company strategy. This project has extremely high specificity and few side effects theoretically, and its effect has a great advantage over traditional therapy. At the same time, RNAi drugs have not been approved in the field of liver cancer, and there are very few gene drugs and target drugs. Therefore, the company should adopt a differentiated strategy to continuously enhance its advantages and1 make it a unique product in the industry, which will increase costs, but lead to the high quality and effectiveness.

(3) The price making should consider the patient’s job, family income, and actively apply for medical insurance drugs.

On April 28, 2018, at the press conference of the State Council Information Office, Zeng Yixin, deputy director of the National Health and Health Committee, emphasized the initiation of centralized procurement and medical insurance access negotiations for anti-cancer drugs and improvement of the anti-cancer drug supply security policy. We can enable patients to afford the drug by becoming a medical insurance drug.
Citation

[1] the 15 most common cancers world (W) in 2018 are shown in descending order of the overall age-standardized rate for man Source: GLOBOCAN 2018.


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