Gene Therapy in China

From a Dutch perspective

2010

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This project was executed by Dr. Leonie C.M. Kaptein. She received important input from Chinese resources with the help from Drs. Yuedan Li. They are working in the group of Prof. Dr. Gerard Wagemaker, department of Hematology, Erasmus Medical Center, Rotterdam.

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SAMENVATTING

Achtergrond

De COGEM heeft in 2006 in haar signalering over de ontwikkelingen van gentherapie in China de verwachting uitgesproken dat dergelijke ontwikkelingen een grote vlucht zou kunnen nemen en dat Nederland in de nabije toekomst met Chinese gentherapeutica geconfronteerd zou kunnen worden. De huidige studie was geïnitieerd door de COGEM om informatie te verkrijgen uit de Chinese wetenschappelijk literatuur over de ontwikkelingen betreffende gentherapie. Speciale aandacht hierbij zou moeten uitgaan naar verkrijgen van gegevens over mogelijke milieurisico's.

Ontwikkelingen gentherapie

Groei van gentherapieveld in China

Analyses van Chinese literatuur databases (CNKI, Wanfang and CQVIP) en Westerse literatuur databases (ISI Web of Science, Pubmed) laten een toename zien van publicaties, over gentherapie, afkomstig van Chinese auteurs. Daarnaast treedt er een verschuiving op van publicaties in Chinese databases naar Westerse literatuur databases.

Onderzoeksactiviteiten zijn net zo divers als in de rest van de wereld

- De onderzoeksonderwerpen in China zijn net zo breed als in andere landen. Er is wel een duidelijke focus op ziekten die een relatief hoge prevalentie in China hebben, zoals lever gerelateerde ziekten. Analyses van de CNKI database laten een prominent aandeel van kanker zien (50%), gevolgd door lever gerelateerde aandoeningen (11%), neurologische aandoeningen (11%), cardiovasculaire aandoeningen (6%) en diabetes (2%).
- Non-virale technieken (m.n. RNA technologiën en lipofection) worden steeds meer beschreven (43% van de studies). Adenovirale vectoren zijn nog steeds populair (17%). Conventionele retrovirale vectoren worden duidelijk minder bestudeerd (4%), lentivirale vectoren (2,8%). Herpes simplex virus (1.6%), poxvirus (0,1%), Sendai virus (0,1%), vaccinia virus (0,1%) worden slechts beperkt bestudeerd.

Klinische studies nemen afgelopen jaren toe

- Totale aantal gevonden klinische studies die goedgekeurd zijn in China bedraagt 44. De eerste studie was reeds goedgekeurd in 1991, echter, volgende studies werden relatief minder snel gestart in vergelijking met andere landen (6 studies in totaal tot 2000, terwijl er vanaf 2000 een toename te zien is (2-6 studies per jaar).
- De meerderheid van de klinische studies betreffen kankerpatiënten. Andere ziekten betreffen cardiovasculaire aandoeningen, ischemie, hepatitis B en hemofilie B.
- Anekdotische informatie is gevonden van 5 Nederlandse patiënten die Gendicine® behandeling hebben ondergaan in China. Verder is door een Chinese dokter aan een Nederlandse patiënt gesuggereerd om Gendicine® behandeling te ondergaan in Nederland. Verschillende websites zijn gevonden die als duidelijk doel hebben om buitenlandse patiënten aan te trekken voor gentherapie behandeling in China.
- Het totale aantal patiënten die gentherapie behandeling ondergaan in China is veel hoger dan in de rest van de wereld door de registratie van Gendicine® en Oncorine®. Deze producten zijn ook in gebruik voor off-label toepassingen.
- Tenminste tien bedrijven in China zijn actief in het gentherapie veld en recent zijn er nog twee bedrijven opgericht. Vijf andere bedrijven zijn gericht op genetische vaccines. Een ander bedrijf, Chongqing Zongsheng Biotech (Chengdu), heeft sinds 2006 Tiefu™ (Ad-CTLA4Ig in varkenshuidcellen) op de markt gebracht voor de behandeling van huidwonden.
- Er kan geconcludeerd worden dat klinische activiteiten in China sneller toenemen dan in de rest van de wereld. Er zijn meer bedrijven actief dan tot nu toe bekend was en zij beslaan een breed spectrum van activiteiten.

Milieurisico's

Waarnemingen die tot nu toe deels nieuw zijn voor China

- Medisch toerisme voor gentherapie in China is compleet nieuw fenomeen. Ofschoon het aantal patiënten dat afgereisd is vanuit Nederland naar China relatief laag is, kunnen de consequenties veel grotere reikwijdte hebben. Deze kunnen namelijk niet alleen de behandelde persoon betreffen, maar in potentie ook de omgeving van dit individu. Zeker wanneer patiënten hun alternatieve behandeling niet bespreken met hun reguliere behandelaar(s) en het gentherapeuticum gecombineerd gaat worden met andere behandeling(en) in Nederland zonder juiste kennis. Vanzelfsprekend zal de eenvoudige toegang via internet om nieuwe behandelingen te kunnen verkrijgen zal serieus genomen moeten worden.

- De vernieuwende wondbehandeling met Tiefu™ die tot nu toe onopgemerkt was gebleven, laat zien dat de bredere toepassing van virale vector technologie inmiddels in de praktijk is gebracht. Dit product wordt beschouwd als een medisch hulpmiddel en valt officieel niet onder gentherapie. Het gebruikte insert, CTLA4Ig, heeft een immuun onderdrukkend effect. Vanzelfsprekend is het onwenselijk om genen in het milieu te introduceren die een remmend effect kunnen hebben op het immuunsysteem van de algemene bevolking.
- China heeft de potentie om het land te worden met de meeste klinische data van patiënten behandeld met gentherapie. Documentatie en publicatie van deze data, inclusief shedding data, zou verder verbeterd kunnen worden om toekomstige risico analyses beter te kunnen onderbouwen.

Overige waarnemingen relevant voor risico analyses

- Er is gesuggereerd dat de dood van Jesse Gelsinger in 1999 in the VS geen effect teweeg heeft gebracht in China. Echter, zijn tragische dood leidde tot de organisatie van de Beijing Xiangshan Summit Conference over gentherapie. Er was algemene consensus in de Summit dat ethische toetsing een cruciale stap is niet alleen voor ter bescherming van patiënten, maar ook van belang is om de naam van de natie hoog te houden.
- De toepassing van Tiefu™ draagt niet alleen een risico van ongewenste virus transmissie van de gebruikte adenovirale vector, maar door het gebruik van varkenscellen is dit een interventie die kan leiden tot overdracht van dierlijke virussen naar de mens. Xenotransplantatie (zoals eilandjes van Langerhans om type I diabetes te behandelen) wordt ook onderzocht in andere landen. De mogelijkheid van virus transmissie van dier naar mens is derhalve niet uniek voor China, maar blijft zeker een belangrijk onderwerp om te blijven volgen zeker indien het gaat om genetisch gemodificeerde xenotransplantatieproducten.

Conclusie

De informatie over gentherapie in China zoals beschreven in dit rapport geeft de huidige stand van zaken, maar kan de komende vijf sterk verder ontwikkeld zijn. Het is derhalve belangrijk om verdere ontwikkelingen te blijven volgen.

SUMMARY

Background

In 2006, The Netherlands Commission on Genetic Modification (COGEM) has reported on "Developments of gene therapy in China" and indicated that these developments could have profound consequences for gene therapy worldwide, including The Netherlands. The present study was initiated by COGEM to retrieve information from Chinese sources, including the scientific literature relating to gene therapy. Special focus should be on possible risks for the environment.

Developments in gene therapy field

Maturation of the gene therapy field in China

Analyses of Chinese literature databases (CNKI, Wanfang and CQVIP) and Western literature databases (ISI Web of Science, PubMed) showed an overall increase in number of publications, on gene therapy, by scientists from China as well as a shift to publications in non-Chinese and also in Western literature databases.

Research activities are as broad as in the rest of the world

- In terms of topics, research in China is as broad as in the rest of the world. A prominent focus is apparent to disease areas that have a relative high prevalence in China, e.g., liver related disorders. Analyses of the CNKI database displayed the prominent position of cancer (50%), followed by liver related disorders (11%), neurological disorders (11%), cardiovascular diseases (6%), and diabetes (2%).
- Non-viral approaches (especially RNA technologies and lipofection) are described in 43% of the studies. The adenoviral vectors are still popular (17%). Conventional retroviral vectors are studied significantly less (4%), lentiviral vectors (2.8%). Herpes simplex virus (1.6%), poxvirus (0.1%), Sendai virus (0.1%), vaccinia virus (0.1%) are hardly studied.

Clinical studies are increasing in recent years

- The total number of clinical studies approved in China that could be retrieved was 44. The first trial was already approved in 1991. However, the next studies were started at a relative slower rate when compared to the rest of the world (up to 2000 6 studies in total) and showed an increase from 2000 onward (2-6 studies per year).
- The majority of clinical studies concern cancer patients. Others include cardiovascular and ischemic diseases, hepatitis B, and hemophilia B.
- Anecdotal information has been found on five Dutch patients that received Gendicine® treatment in China. It was also suggested by a Chinese doctor to a Dutch patient to be receive Gendicine® treatment in The Netherlands. Several websites have been found that have the clear aim of attracting foreign patients for gene therapy treatment in China.
- The total number of patients undergoing gene therapy treatment in China is much higher than in the rest of the world, due to the registration of Gendicine® and Oncorine®. These products have also been in use for off-label applications.
- At least ten companies in China are directed at gene therapy and recently two more have been established. Five companies are directed at genetic vaccines, another company, Chongqing Zongsheng Biotech (Chengdu), has launched Tiefu™ (Ad-CTLA4Ig expressed in pig skin cells) in 2006 for the treatment of skin wounds.
- It is concluded that the clinical activities in China increasing faster than world-wide. More companies are active than were known so far and are covering a broad spectrum of activities.

Safety issues

New type of issues that are in some aspects unique for China up to now

- The issue of medical tourism for gene therapy treatment in China is unprecedented. Even though the numbers of patients that have traveled to China appear to be relatively low, gene therapy treatment could not only have consequences for the treated individual, but also potentially harbor risks to the environment of the treated individual, although small. Especially when patients do no communicate the alternative treatment to their regular physician(s) and the gene therapeutics are combined with therapy in The Netherlands without proper knowledge. Obviously, the easy access by internet to obtain new type of treatments has to be taken seriously.
- The innovative biological wound dressing Tiefu™ had gone unnoticed and demonstrates that broader application of viral vector technology has become a reality. This product is considered a medical device and is not defined as gene therapy. The used transgene, CTLA4Ig, has an immune suppressive effect. Obviously, it would be undesirable to introduce genes into the environment that could exert an inhibitory effect on the immune system of the general population.

- China has the potential to become the country with the most clinical data from gene therapy patients. Recording and publication of these data, including shedding data, should be further improved to enable future risk assessments. Up to now shedding data have only been limited reported in scientific articles.

Other noteworthy topics related to risk assessment

- It has been suggested that China was not affected by the death of Jesse Gelsinger in 1999 in the USA, however, this tragic death resulted in the organization in the Beijing Xiangshan Summit Conference on gene therapy. There was a general consensus in the Summit that ethical review is a crucial step not only to protect the human subjects, but also to protect the reputation of the nation.
- The application of Tiefu™ does not only harbor some risk of undesired viral transmission of the applied adenoviral vector, but the use of pig skin entails a cross-species intervention with other specific risks and should be done with caution to prevent transmission of animal viruses to man. Xenotransplantation (e.g., pancreatic islet cell transplantation to treat type I diabetes) is also investigated in other countries. The issue of transmission of viruses crossing the species barrier is therefore not unique for China, but still is an important issue in view of the registration of a gene modified xenotransplantation product.

In conclusion

The information on gene therapy in China described in this report reflects the current state of the art, but might change rigorously in another five years time. Therefore, continued monitoring of its further development will be essential.

BACKGROUND REPORT

On May 4, 2006, the Netherlands Commission on Genetic Modification (COGEM) reported to the Ministry of Housing, Spatial Planning and the Environment ongoing "Developments of gene therapy in China". This interest was initially fueled by the 2003 approved registration of Gendicine® (recombinant human Ad-p53 injection) by the Chinese State Food and Drug Administration (SFDA). Gendicine® became the first gene therapeutic medicine in the world, registered for the treatment of head and neck squamous cell carcinoma. Also in China, the second registered gene therapeutic followed in 2005: Oncorine® became the first approved oncolytic adenovirus for the treatment of head and neck squamous cell carcinoma.

The estimated numbers of patients to be treated by Gendicine® before the end of 2006 was as high as 50.000. Therefore, it was anticipated that these Chinese gene therapeutics might have profound consequences for gene therapy worldwide, including The Netherlands. Indications of medical tourism raised questions on safety aspects not only for the individual patient as well as for the environment. Potentially, the treated patients could spread the viral vector to their immediate environment, including, e.g., family members, friends or health care providers.

The present study was initiated by COGEM to retrieve information from Chinese sources, including the scientific literature relating to gene therapy and shedding data, with the thought that such a study would enable a more proactive stance to the Chinese developments. Such data are difficult to obtain and to assess without proper knowledge of the Chinese language. The obtained information would be a valuable basis to further monitor the ongoing developments in China and thereby enable anticipation on new registrations, new vector technologies and related safety risks. In addition, information on shedding data would be most welcome for future risk assessments based on experiences from clinical settings.

The study was initiated to fill the existing knowledge gap by actively obtaining data from Chinese resources. Initial exploration of Chinese literature databases and consultation with several Chinese scientists indicated that literature analysis alone might not be sufficient to fulfill the goal of this project. The obtained data were expected to be only a subset of the ongoing activities. This project was executed by Dr. Leonie C.M. Kaptein. She received important input from Chinese resources with the help from Drs. Yuedan Li. They are working in the group of Prof. Dr. Gerard Wagemaker, department of Hematology, Erasmus Medical Center, Rotterdam.

This report starts with a chapter that places the development of gene therapy in the broad perspective of Chinese biomedicine and developments elsewhere, followed by a chapter with detailed information on Chinese literature databases. Chapters 3 and 4 provide a summary of clinical studies and research activities, respectively. An overview of companies and international collaborations is described in Chapter 5. A separate chapter is devoted to safety issues, including shedding data, cross-species medical interventions and medical tourism. Overall observations and conclusions are given in Chapter 7. The report concludes with relevant websites listed in Chapter 8.

The information contained in this report is relevant for regulatory authorities (assessment of risks to the environment possibly introduced in The Netherlands by patients treated in China, as well as anticipating new developments and suggestions for future monitoring), researchers and companies (overview of relevant scientific and drug developments), clinicians (additional background knowledge and awareness of novel gene therapy treatments), and, last but not least, for patients (alertness on efficacy and safety of the new treatment modalities).

For this project three meetings were organized with the supervisory committee set up by the COGEM to assess the focus of the study and to provide feedback on the contents of the report.
We gratefully acknowledge the members of the supervisory committee:

- Prof. Dr. Rob Hoeben, Department of Molecular Cell Biology, LUMC, Leiden, The Netherlands
- Dr. Rik Bleijs, Gene therapy office, RIVM, Bilthoven, The Netherlands
- Prof. Dr. Winald Gerritsen, Cancer Center Amsterdam, Amsterdam, The Netherlands
- Prof. Dr. Geke A.P. Hospers, Department of Medical Oncology, UMCG, Groningen, The Netherlands
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This project was executed by Dr. Leonie C.M. Kaptein and input from Chinese resources was provided by Drs. Yuedan Li, both working in the research group headed by Prof. Dr. Gerard Wagemaker, Department of Hematology, Erasmus Medical Center, Rotterdam.

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1. DEVELOPMENTS OF GENE THERAPY IN PERSPECTIVE

From "made in China" to "discovered in China"

The title of this section is taken from the GlaxoSmithKline (GSK) website / R&D in China. In the past 20 years, GSK has invested over RMB1 billion (more than 100 million EURO) in R&D in China. In 2007, a global R&D centre was set up in Shanghai to focus on research into neurodegeneration in order to create new medicines for profound medical needs such as multiple sclerosis, Parkinson's disease and Alzheimer's disease. The phrase ' From "made in China" to "discovered in China" ' will hold true for the ambitions of GSK, however, many people around the world will still be skeptical about this transformation from within China and will regard China as a true 'copy cat' nation. However, the past contributions of China to biological sciences have already proven otherwise.

Within the Human Genome Project, China, as one of six member states, played a minor role by completing the sequencing of the region between 3pter and D3S3397 of human chromosome 3, also referred to as the "Beijing region" (Luo, Li et al, 2005). The complete sequencing of the rice genome was the accomplishment of scientists in China. In fact, major achievements were already published in 1963 by the late embryologist Dizhou Tong who transferred nuclear DNA from a cell of a male Asian carp to an egg of a female Asian carp and thereby produced the world's first cloned fish. This was the first time that such a complex organism was cloned. Previously, this had only been achieved for microorganisms and nematodes, as well as amphibians. Ten years later, Dizhou Tong also created the first interspecies clone of a carp. See also Table 1.1 for an overview of various highlights of biological sciences in China over time.

Table 1.1 Highlights of biological sciences in China (*in italics: for reference: relevant highlights in rest of the world*)

1963	nuclear DNA transfer from a cell of a male Asian carp to an egg of a female Asian carp: world's first cloned fish
1973	nuclear DNA transfer of an Asian carp into the egg of a European crucian carp: world's first interspecies clone
1990	*Birth of Herman, world's first genetically modified bull [female descendants would produce milk with human lactoferrine*]
1997	*Birth of Dolly, world's first cloned sheep, also first cloned mammal [cell of the udder fabric of an adult ewe was used to generate Dolly]*
2002	*Completion of sequencing of the human genome* – China contributed to the Human Genome Project by sequencing the "Beijing region" of chromosome 3
2002	Completion of sequencing of the rice genome

Two major hallmarks relating to gene therapy in China are (1) the first gene therapy clinical trial in the world for hemophilia in 1991 and (2) the first registered gene therapeutic medicine in the world, Gendicine for head and neck squamous cell carcinoma, by Shenzhen SiBiono Gene Tech in 2003. At first, the accomplishments were received with great interest, however, soon both milestones were surrounded by debate and controversies. The clinical trial appeared to show several scientific flaws and the registration was merely based on safety rather than efficacy data. In addition, Gendicine is very similar to Advexin (from Texas based Introgen Therapeutics, licensed from the M.D. Anderson Cancer Centre in 1993). Also the second registered gene therapeutic medicine in the world, Oncorine, was registered in China. No other gene therapeutic medicines have been registered so far. The Netherlands has also been a player in the gene therapy field from the beginning. In 1991 the first clinical protocol in The Netherlands was approved for gene therapy of adenosine deaminase deficiency. Dutch scientists generated the PER.C6 cell line for the production of replication defective adenoviral vectors (Fallaux et al, 1998). This human cell line became the cornerstone on which Crucell was built and is now used as a platform for producing new viral vaccines. In late 2009 Amsterdam Molecular Therapeutics (AMT) filed its main product, Glybera, for treatment of lipoprotein lipase deficiency to EMA (European Medicines Agency) and is expecting their opinion early 2011. Furthermore, in 2009 GlaxoSmithKline (GSK) and Prosensa, the Dutch based biopharmaceutical company focusing on RNA modulating therapeutics, announced their collaboration for the development and commercialisation of RNA based therapeutics for Duchenne Muscular Dystrophy (DMD). The financial terms include a 18 million euro upfront payment by GSK. Furthermore, Prosensa is eligible to receive up to 480 million euro in milestones payments if their 4 compounds are developed successfully.

Up to now, it is hard to find convincing clinical data from gene therapy studies performed in China. As a consequence, the gene therapy community has taken a cautious attitude towards China. Still, there is also positive news from China in the medical field. Xianling Gubao, the leading traditional Chinese medicine (TCM) for the treatment of osteoporosis in China, is now in phase 4 clinical trials in the USA. This medicine is the flagship product of Shenzhen based Tongjitang Pharmaceutical Company, which is working in collaboration with Synarc (San Francisco) and the Department of Epidemiology and Biostatistics Lab at the University of California, San

Francisco to seek registration of the product in the US market. This study is one of the first evidence-based clinical studies on the efficacy and safety of TCM with proven results according to US Food and Drug Administration guidelines (Frew et al, 2008).

Taken together, it becomes clear that China has the ambition to make a difference in the (bio)medical field. The first registered gene therapeutic medicine is registered in China and the number of patients undergoing gene therapy treatment in China is currently bypassing the number of patients elsewhere in the world. Even though this product in fact falls in the category of 'copy cat', it is still fair to conclude that in the future China might prove that it can not only make gene therapeutics, but that it will also be able to 'discover' those.

Trends over time

In order to follow the developments in China more closely it would be helpful to have reliable and convenient data sources. Talking to various Chinese scientists either within or outside of the gene therapy field, it turns out that Chinese researchers themselves prefer to use the PubMed database in order to check for the latest data in their respective fields. They all indicate that when relevant work is performed in China, the authors prefer to publish in English since this is regarded as a higher standard. Looking for literature in the gene therapy field that is published by scientists from China shows a remarkable increase in the last few years. The ISI Web of Science was used for analyzing the number of publications on gene therapy that have been published by different countries over the years 2000-2009. Results show a striking increase for China as compared to all other countries (Figure 1.1). China published nearly 9 times more articles in 2009 than in 2000. The Netherlands is following at a great distance together with Spain and South Korea as countries with a ratio of around 2-3

fold increase. With the exception of Italy with a 1.3 fold increase, no other countries show an increase in gene therapy publications over the last decade. Only the USA is still publishing in absolute numbers a greater number of articles, i.e. more than 3 times the number of articles published by China. However, the numbers from USA have declined by more than 20% in the last 10 years. These trends over time indicate that China is going to play a more significant role in the gene therapy field as compared to previous years. In order to put the numbers in perspective publications on "gene therapy" were normalized to the gross domestic products (GDP) of the respective countries for the year 2009 as shown in Figure 1.2. China then takes place number 8 directly behind the USA, whereas The Netherlands take the lead in this comparison. More in-depth analyses of publications from Chinese authors will shed further light on contributions from China so far. This is described in more detail in the next chapter - analyses of databases as well as chapter 4 – research activities.

As a comparison, similar analyses were done for publications in the stem cell field (Figure 1.3). In this area the relative increase is much higher, up to nearly 35 fold for China. Remarkably, China is closely followed by South Korea. Other countries including The Netherlands show a 3-5 fold increase. The UK, despite having a strong tradition in stem cell research, shows no increase in publications since 2007. In addition, Italy and Japan show a decline in publications in the last year. Again, the USA is publishing in absolute numbers the most articles, nearly 4 times over the number of articles published by China, but the number of articles in 2009 has dropped slightly from the previous year. Thus, for both the stem cell and gene therapy fields the roles of the different players could be expected to change in the near future, with China moving into a more prominent role.

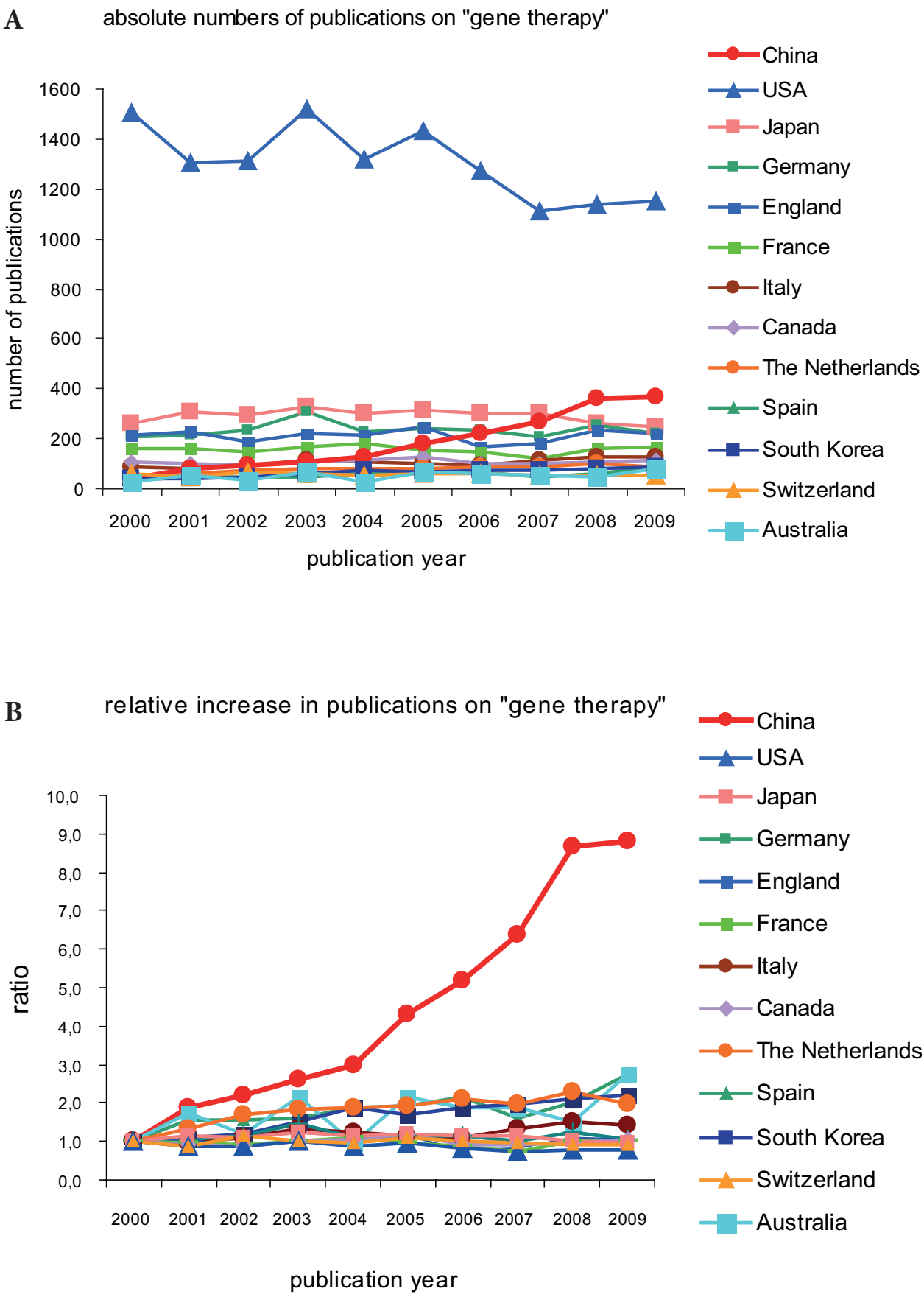


Figure 1.1 Gene therapy publications per year by country for 2000-2009.
Data were obtained by using "gene therapy" as a search topic for the years 2000-2009 and refining results by country using the ISI Web of Science citation index expanded database. **A** shows the absolute numbers and **B** relative increase in publications with the year 2000 as a reference (1.0).

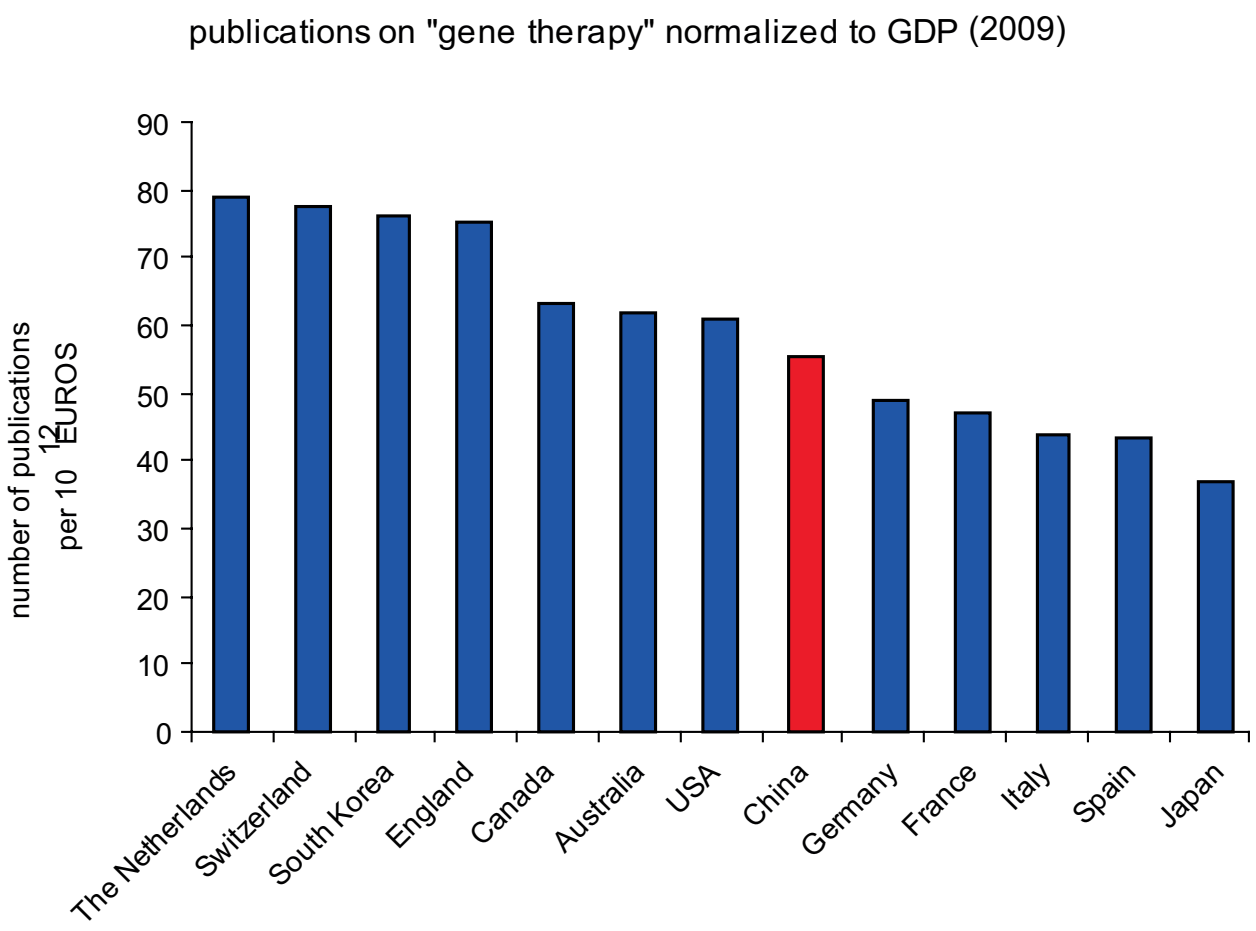


Figure 1.2 Publications on "gene therapy" normalized to the gross domestic products (GDP) of the respective countries for the year 2009. Data were obtained by using "gene therapy" as a search topic for the year 2009 and refining results by country using the ISI Web of Science citation index expanded database, September 2010. The GDP data for the year 2009 were obtained from the International Monetary Fund, World Economic Outlook Database, October 2010.

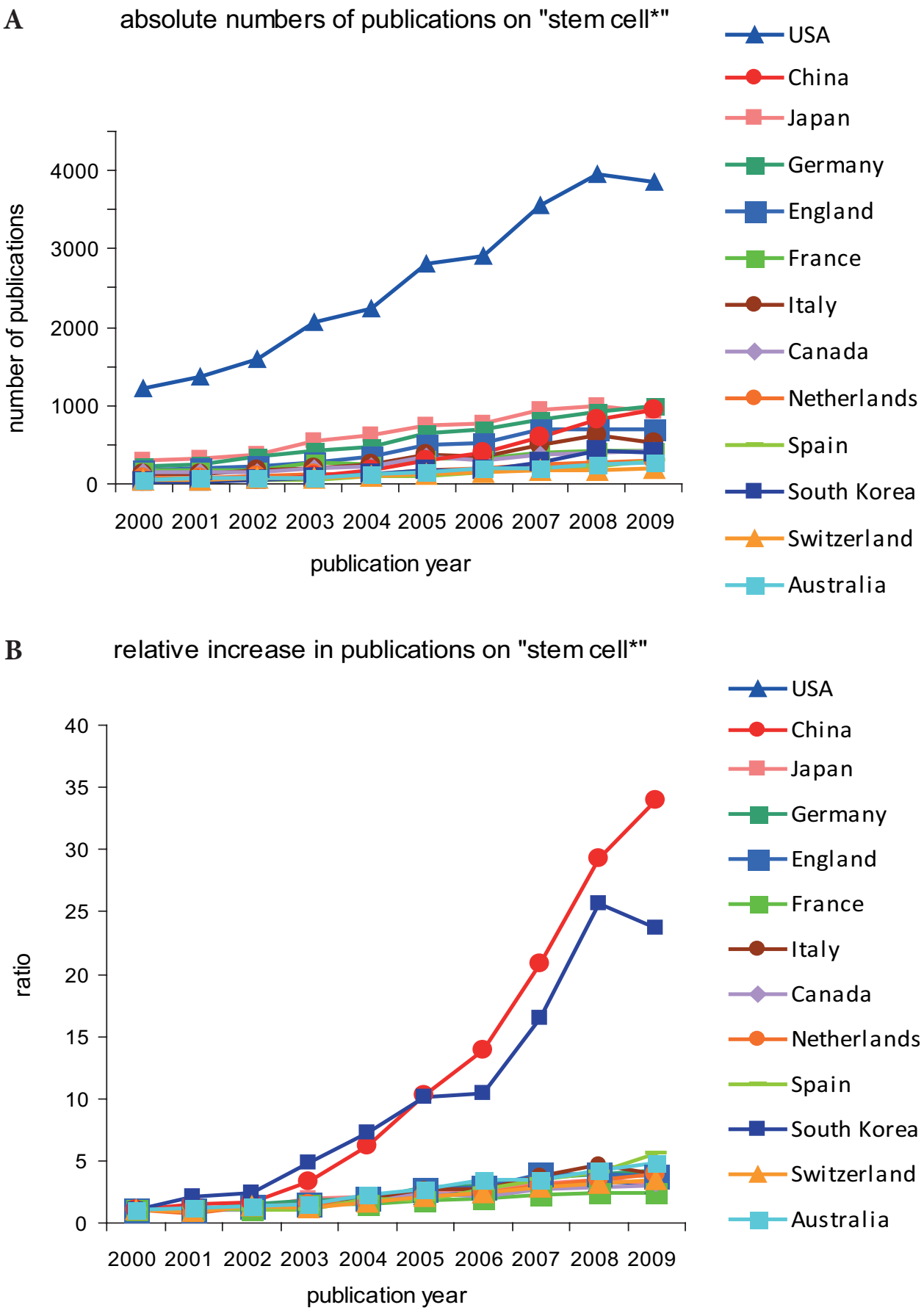


Figure 1.3 Stem cell publications per year by country for 2000-2009.
Data were obtained by using "stem cell" as a search topic for the years 2000-2009 and refining results by country using the ISI Web of Science citation index expanded database. **A** shows the absolute numbers and **B** shows the relative increase in publications with the year 2000 as a reference (1.0).

Medical tourism

Publicity on promising new medical treatments always captures the interests of patients who are in desperate need of finding a therapy for their disease or who have not yet been treated satisfactorily. Major attention has been drawn to stem cell treatments offered in China. A nice comprehensive overview of stem cell-based clinical trials in China is written by Lianming Liao and Robert Chunhua Zhao (Stem Cells and Development, 2008).

Stem cells that were not well characterized were used in some hospitals. Unlike common somatic cells, when used as a new therapeutic modality, Adult Stem Cells (ASCs) do not require approval from the State Food and Drug Administration (SFDA) of China, the counterpart of the US FDA. Thus, almost all clinical trials with stem cells in China have not applied for approval from the SFDA. Approval is usually obtained from Ethics Committees of the institutes and hospitals and sometimes registered their trials in the local health care authorities. Since the safety and efficiency of stem cell-based clinical trials cannot be guaranteed without the monitoring of the SFDA, concerns have been expressed by experts in the stem cell field (Stephenson 2008). The International Society for Stem Cell Research recently issued a press release on their concerns on stem cell tourism (www.isscr.org/press_releases/clin_guidelines.html).

The number of patients travelling to China for stem cell treatment has been estimated at 2000 (McMahon et al 2010).

Compared to stem cell treatment in China, the situation for gene therapy treatment is somewhat different. Clinical trials involving gene therapy have to be approved by the SFDA. Even so, two medicines (Gendicine and Oncorine, both based on adenoviral vectors) have already been registered by the SFDA. However, these registrations have been questioned. Approval seems to be given on the basis of tumor shrinkage, rather than extension of patient lifetime. There has been quite some concern from gene therapy researchers elsewhere in the world as to the quality of the trials performed and thereby the safety and efficacy of the treatment (Edelstein, 2007). Still, these medicines are now available in China. Estimates of patient numbers travelling to China to receive Gendicine treatment is estimated at 300-600 (dr. Peng, Shenzhen Sibiono GeneTech). The estimated numbers of medical tourism in the field of gene therapy are approximately a factor 3-5 fold lower that in the field of stem cells. Furthermore, up to now there has been no official statement from a Society for Gene Therapy on concerns related to gene therapy tourism.

How the field will develop further is difficult to predict, but from figures 1.1 and 1.2 it could be concluded that the expansion of activities in the fields of gene therapy and stem cells will continue in China. In addition, it is important to realise that gene therapy treatments offered by Chinese companies could become more accessible outside of China, whereas stem cell treatments will most likely be offered only in China due the more complex nature of the treatments. Gene therapeutic medicines may have broader consequences that could have implications for clinical practice in Western countries. Taking all of these aspects together gives ample grounds for further monitoring of the gene therapy and stem cell activities that will be pursued in China in the upcoming years.

2. ANALYSES OF DATABASES

Chinese literature databases

The main goal of this project was to retrieve information from scientific literature published by Chinese authors relating to gene therapy and shedding data. In the first chapter the ISI Web of Science database was used in order to make a convenient comparison of gene therapy publications output between different countries over the last 10 years. It is of interest to know how the numbers in Western literature databases relate to Chinese literature databases.

To this aim three different databases were used: the Wanfang database, the Chongqing Vip Chinese Scientific and Technical Periodical (CQVIP) database and the China National Knowledge Infrastructure (CNKI) database. (1) The Wanfang database is affiliated to the Chinese Ministry of Science & Technology and considered to be the leading information provider in China since 1950s. With a wide range of database resources and value-added servi-ces, Wanfang Data has become a gateway to understand Chinese culture, medicine, business, science, et cetera. (2) The CQVIP database was established in 1993 and has collected titles, abstracts and full texts of 12,000 Chinese journals on biology, agriculture, and medicine et cetera since 1989. It provides comprehensive information and the contents is being updated quarterly. This database is available after sign up and purchase. (3) The China National Knowledge Infrastructure (CNKI) is a key national project of China started in June 1996 for knowledge sharing throughout China and the world and has grown substantially over the years. Obviously, for searching original articles in Chinese one needs know ledge of the Chinese language. However, one has to realise that not all Chinese journals publish in Chinese, as some Chinese journals are published in English. Website addresses and short additional information on these three databases are given in Chapter 8: Relevant websites.

Figure 2.1 shows the numbers of publications on gene therapy as obtained from the three different databases for the years 2000-2009. For the Wanfang and the CQVIP databases "gene therapy" could only be used as a search term in 'title' and/or 'key words' but do not provide an option to search in a broader field as 'topic'. For the CNKI database "gene therapy" was used as search term in 'title' and/or 'key words' or in 'topic' (separate lines in graph). The CQVIP database shows a steady number of publications (approximately 750 per year), this is however declining to 551 in 2008 and 355 in 2009. The Wanfang database shows an increase in number of publications, from 390 in 2000 to 1127 in 2007, however, also from 2008 on a decrease in number of publications

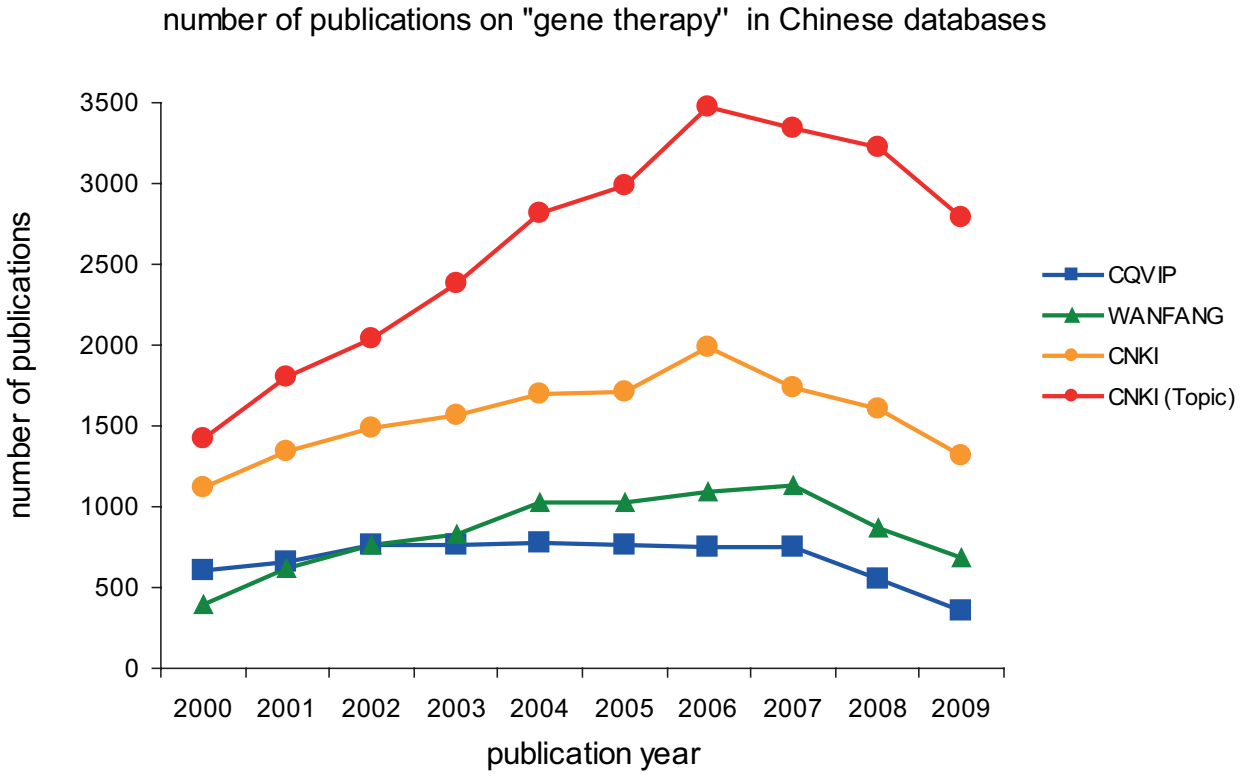


Figure 2.1. The number of publications on gene therapy per year as found in the Wanfang database, the CQVIP database and the CNKI database respectively. For the first 2 databases "gene therapy" could only be used as a search term in 'title' and/or 'key words'. For the CNKI database gene therapy was used as a search term in 'title' and/or 'key words' or in 'topic' (upper line). *(analyzed September 2010).*

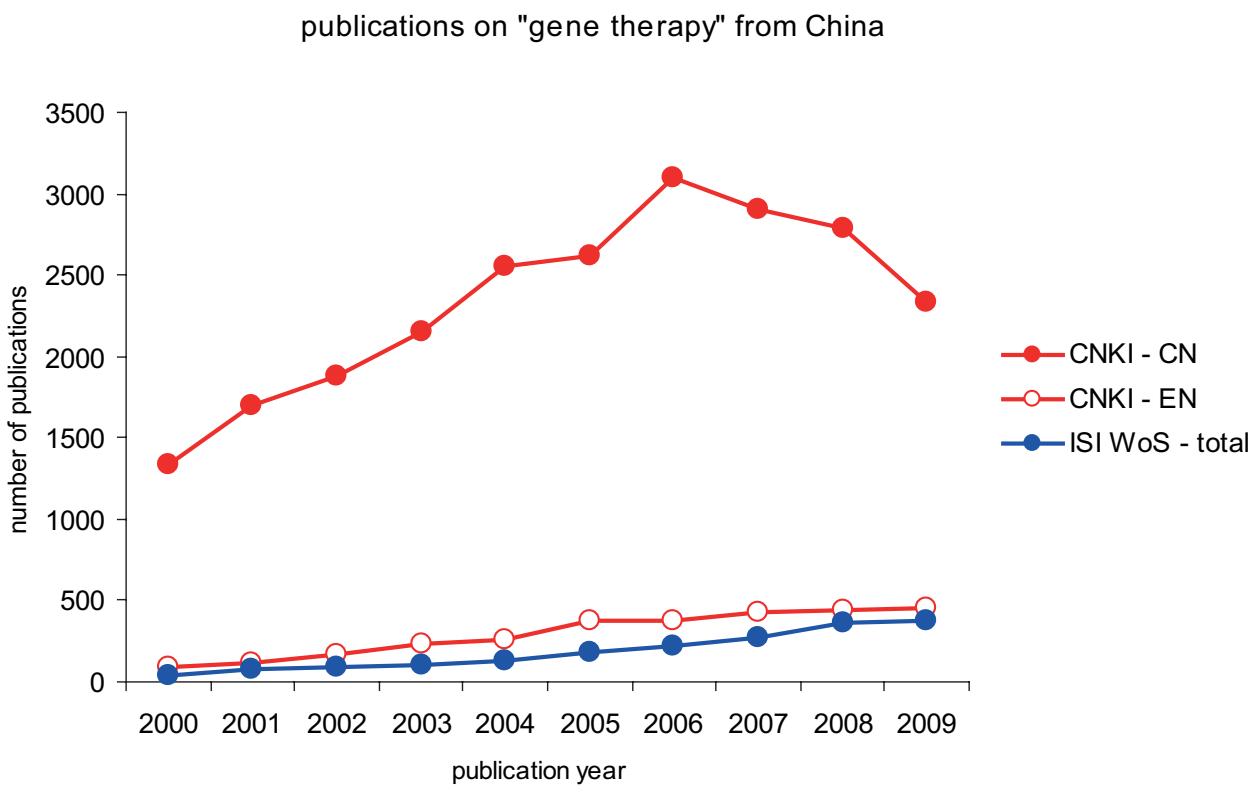


Figure 2.2. Gene therapy publications per year as found in the CNKI database and the ISI Web of Science, using "gene therapy" as a search term in 'topic'. CNKI – total refers to the total number of publications retrieved, CNKI – EN refers to the number of publications retrieved that have been published in English. The graph of ISI WoS show the same data as given in Figure 1.1 *(analyzed September 2010).*

could be observed (870 in 2008 and 690 in 2009). The CNKI database yields the highest number of publications, starting with 1113 in 2000 rising to 1989 in 2006, after which a decline in number of publications is seen over the following years. The highest number of articles (3096 in 2006) could be retrieved from the CNKI database by using "gene therapy" as a search item in 'topic', which is the same search strategy that was used for retrieving gene therapy articles from the ISI Web of Science database, as shown in Chapter 1, Figure 1.1. This figure showed an approximately 3-fold increase in publications of Chinese authors on gene therapy from 2000 to 2004, whereas from 2005 the number increase at a somewhat more rapid speed up to 2008 to stabilize in 2009. It is now interesting to note that the expansion of scientific output observed in the ISI Web of Science database is not seen in the Chinese databases after 2006, instead a declining trend has been observed in the most recent years. It can therefore be concluded that there is a shift in preferences by Chinese scientists in favour of publishing in English language journals. This indicates that articles on gene therapy from Chinese authors are now becoming more easily available to Western scientists.

Accessibility of original research papers

The most informative database, i.e. the CNKI database, rendering the most publications as well as having options for most practical search strategies, was subsequently used for all other additional analyses. Figure 2.2 shows the gene therapy publications per year as found in the CNKI database, showing separate graphs for all publications retrieved as well as the publications retrieved that are written in English language. For the articles in English, it is difficult to separate these in articles from journals published in China and in articles from journals published outside China. For further comparison the total numbers of publications from Chinese authors as obtained from the ISI Web of Science database have been shown in the same graph. The absolute numbers are actually quite similar for both databases, the numbers in the CNKI database are only slightly higher than the numbers obtained in the ISI Web of Science. The publications will be in part actually the same publications, however, it is difficult to check what percentage is exactly the same.

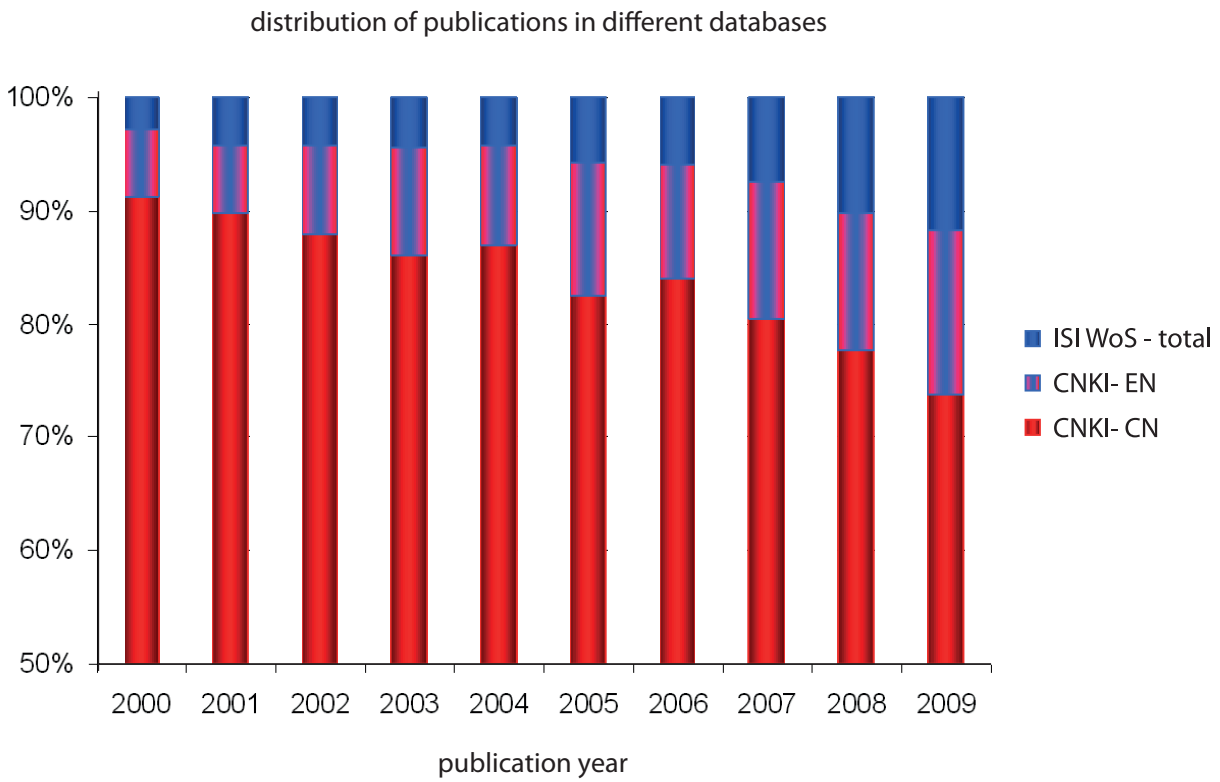


Figure 2.3. The relative distribution of the publications from China in different databases. For searching the databases "gene therapy" was used in 'topic'. (analyzed September 2010). Data in blue are based on searches in the ISI Web of Science, data in red are based on searches in the CNKI database and represent the publications in Chinese language, data in red/blue are based on searches in the CNKI database and represent the publications in English language. In case both databases cover all publications from Chinese authors, the percentage coverage for each database will then be 50%.

Figure 2.3 shows what fraction of the publications from Chinese authors are now easily available to the Western world and what part will still be missed. In 2000, only 3% of the articles written by Chinese scientists on gene therapy could be retrieved. As in 2009, this percentage has grown to 14%. This percentage could still be an underestimation since part of the publications that are listed in the CNKI database will actually be overlapping with the Western databases, i.e. the publications in English. Of the total number of publications by Chinese authors published in 2009 69% was in the Chinese language. Since the overall number of publications retrieved from the CNKI database are still a large proportion of the Chinese scientific output, these publications have been studied in more detail. Information on the scientific content has been given in chapter 4 – research activities. Below additional information is given on journals and affiliations of the authors.

Representation of journals listed

When analyzing scientific output, not only quantity but also quality matters. In order to get some insight on this it is interesting to know in what journals the articles from Chinese authors have been published. A closer look at the ISI Web of Knowledge / Web of Science database furthermore reveals that the top 20 list of journals in which Chinese scientists publish as retrieved in this Western database, actually lists 6 Chinese journals (even already listed as number 1 and 4, see Table 2.1). This shows that we in fact are also already looking into Chinese resources. Besides the journals devoted to gene therapy, general medicine and cancer, it is noteworthy to mention that the World Journal of Gastroenterology is listed as number 2 and the Journal of Gastroenterology and Hepatology is listed as number 19. For publications on gene therapy not restricted to any country the top 5 journals are all specifically devoted to gene therapy, which will not come as a surprise, interestingly the number 6 journal is Blood and the next journal devoted to special disease area is Cancer Research at number 9 (see Table 2.2). This could be indicative of the disease areas studied most extensively. As might have been expected a prominent focus is apparent to disease areas that have a relative high prevalence in China, e.g., liver related disorders.

Table 2.1 Top 20 journals publishing articles on gene therapy from Chinese scientists. The ISI Web of Knowledge / Web of Science was searched with topic "gene therapy", analyses was done for countries/territories "Peoples R China" and Sources were ranked according to their record counts, number of record counts is listed in (parenthesis). Number of record counts obtained for results worldwide is listed in [brackets].

1	CHINESE MEDICAL JOURNAL (106) [107]
2	WORLD JOURNAL OF GASTROENTEROLOGY (85) [100]
3	CANCER GENE THERAPY (82) [1,250]
4	ACTA BIOCHIMICA ET BIOPHYSICA SINICA (63) [63]
5	CANCER BIOLOGY & THERAPY (61) [147]
6	GENE THERAPY (59) [2,150]
7	PROGRESS IN BIOCHEMISTRY AND BIOPHYSICS (55)
8	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COM MUNICATIONS (54)
9	ACTA PHARMACOLOGICA SINICA (44)
10	JOURNAL OF GENE MEDICINE (44) [953]
11	CHINESE SCIENCE BULLETIN (36)
12	SCIENCE IN CHINA SERIES C-LIFE SCIENCES (32)
13	HUMAN GENE THERAPY (29) [1750]
14	MOLECULAR THERAPY (29) [1742]
15	BIOMATERIALS (26)
16	INTERNATIONAL JOURNAL OF CANCER (26) [172]
17	CANCER LETTERS (23) [105]
18	JOURNAL OF HUAZHONG UNIVERSITY OF SCIENCE AND TECHNOLOGY-MEDICAL SCIENCES (23)
19	JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY (11)
20	LIFE SCIENCES (11)

Table 2.2 Top 20 journals publishing articles on gene therapy from all countries. The ISI Web of Knowledge / Web of Science was searched with topic "gene therapy", and Sources were ranked according to their record counts. Number of record counts obtained worldwide are listed in (parenthesis), number of record counts obtained for results for countries/territories "Peoples R China" is listed in [brackets].

1	GENE THERAPY (2,150) [82]
2	HUMAN GENE THERAPY (1,750) [29]
3	MOLECULAR THERAPY (1,742) [29]
4	CANCER GENE THERAPY (1,250) [82]
5	JOURNAL OF GENE MEDICINE (935) [44]
6	BLOOD (792)
7	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA (624)
8	JOURNAL OF VIROLOGY (533)
9	CANCER RESEARCH (498)
10	CIRCULATION (465)
11	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COM-MUNICATIONS (322)
12	CLINICAL CANCER RESEARCH (279)
13	CURRENT OPINION IN MOLECULAR THERAPEUTICS (253)
14	CURRENT GENE THERAPY (243) [10]
15	EXPERT OPINION ON BIOLOGICAL THERAPY (236)
16	JOURNAL OF CELLULAR BIOCHEMISTRY (228)
17	JOURNAL OF CONTROLLED RELEASE (227)
18	NATURE MEDICINE (221)
19	ANNALS OF THE NEW YORK ACADEMY OF SCIENCES (143)
20	VIROLOGY (136)

Academic institutes and number of Ph.D. theses

One can easily imagine that the thousands of publications that were retrieved from the CNKI database will be the efforts of many scientists that are working at various laboratories around China. In order to get an overview of the institutes that are currently pursuing gene therapy a search was performed especially for the most recent five years and ranking was done according to the number of publications. The maximum number of affiliations that could be retrieved from the CKNI database is 60, these obtained affiliations account for 56% of the total number of publications in the period 2006-2010. All together gene therapy will therefore be a topic of research at a few hundreds of laboratories around China. The top 20 institutes with the highest output is listed in Table 2.3. Further analyses of the related publications revealed that for many universities, the majority of the publications are Ph.D. theses. The top 3 universities are Jilin University (Changchun, Jilin Province), Shandong University (Jinan, Shandong Province) and Central China University Science and Technology (Wuhan, Hubei Province) respectively with 480 (399), 262 (198) and 259 (202) publications (number of theses and conferences in brackets) as listed in the CNKI database. Only five out of the 20 institutes do not produce any theses, what can be explained by the fact that these are hospitals and therefore are not educating Ph.D. students. From these results it can be concluded that in recent years many Chinese Ph.D. students are trained in gene therapy field and the coming years will learn whether this will lead to an even higher output in scientific articles.

Table 2.3. List of institutes in China that are active in the gene therapy field as can be retrieved from the CKNI database. In yellow institutes that have also been retrieved from the IS Web of Science database [corresponding ranking number is also listed].
The CKNI database was searched with topic "gene therapy", years selected 2006-2010, affiliations were ranked according to their record counts. Total number of publications retrieved: 12234. These publications include theses and/or conferences, the separate numbers for theses and/or conferences are also listed separately. The maximum number of affiliations that can be retrieved is 60, these affiliations account for 55.7% of the total number of publications.

Rank #	Publ	Theses/ Conf.	University, City, Province, Conferences
1 [27]	480	399	Jilin University, Changchun, Jilin Province
2	262	198	Shandong University, Jinan, Shandong Province
3	259	202	Central China University Science and Technology, Wuhan, Hubei Province
4	254	181	Zhongnan University, Wuhan, Hubei Province
5	244	140	Chongqing Medical University, Chongqing
6	213	1	Central China University Science and Technology, Tongji Hospital, Wuhan, Hubei Province
7 [7]	189	129	The Fourth Military Medical University, Xi'an, Shanxi Province
8	182	0	Central China University Science and Technology, Union Hospital, Wuhan, Hubei Province
9	180	124	Zhejiang University, Hangzhou, Zhejiang Province
10	176	128	The Third Military Medical University, Chongqing
11	170	137	Chinese Medical Science University, Shenyang, Liaoning Province
12	160	95	Zhengzhou University, Zhengzhou, Henan Province
13 [9]	159	69	Sichuan University, Chengdu, Sichuan Province
14	154	130	The Second Military Medical University, Shanghai
15	152	1	Sichuan University Huaxi Hospital, Chengdu, Sichuan Province
16 [2]	145	78	Fudan University, Shanghai
17	138	96	Suzhou University, Suzhou, Zhejiang Province
18 [30]	130	96	Tianjin Medical University, Tianjin
19	129	115	Hebei Medical Universtiy, Shijiazhuang, Hebei Province
20	122	0	The Fourth Military Medical University, the First Affiliated Hospital, Xi'an, Shanxi Province

In order to get a better insight on how well the results from these institutes are also accessible to Western scientists, the same ranking was done for institutions retrieved from the ISI Web of Knowledge / Web of Science by searching with topic "gene therapy", analysing for countries/territories "Peoples R China" and ranking Institution Names according to their record counts. The top 20 institutes with the highest output is listed in Table 2.4, the complete list of the 100 retrieved institutes is given in the appendix. Remarkably, the both top 20 lists show only limited overlap (only 4 or 5 institutes out of the top 20). From these data one could conclude that Chinese science is filtered when entering the Western databases. According to Chinese scientists the applied filtering would in fact be a quality indication, thereby giving the impression that we are not really missing out on relevant research developments. This could be confirmed by the fact that the highest number of publications listed in the CKNI database is for authors affiliated to the Chinese Academy of Sciences. In China, this is the most prestigious level that a scientist could achieve. In The Netherlands this could be compared to the Koninklijke Nederlandse Akademie voor Wetenschappen (KNAW).

Table 2.4 List of institutes in China that are active in the gene therapy field as can be retrieved from the ISI Web of Science database. In yellow institutes that have also been retrieved from the CKNI database [corresponding ranking number is also listed].
The ISI Web of Knowledge / Web of Science was searched with topic "gene therapy", analyses was done for countries/territories "Peoples R China" and Institution Names were ranked according to their record counts.

Rank #	Publications (% of total #)	Institution Name
1.	183 (7.97%)	Chinese Acad Sci
2. [16]	147 (6.40%)	Fudan Univ
3.	132 (5.75%)	Huazhong Univ Sci & Technol
4.	126 (5.49%)	Shanghai Jiao Tong Univ
5.	109 (4.75%)	Chinese Acad Med Sci
6.	101 (4.40%)	Zhejiang Univ
7. [7]	97 (4.22%)	Fourth Mil Med Univ
8.	95 (4.14%)	Univ Hong Kong
9. [13]	94 (4.09%)	Sichuan Univ
10.	92 (4.00%)	Peking Univ
11.	84 (3.66%)	Sun Yat Sen Univ
12.	82 (3.57%)	Mil Med Coll 2
13.	78 (3.40%)	Peking Union Med Coll
14.	76 (3.31%)	Shandong Univ
15.	71 (3.09%)	Wuhan Univ
16.	66 (2.87%)	Cent S Univ
17.	55 (2.40%)	Nanjing Med Univ
18.	48 (2.09%)	Harbin Med Coll
19.[?42]	45 (1.96%)	Third Mil Med Univ
20.	43 (1.87%)	Chinese Univ Hong Kong

In conclusion

From these data, we may conclude that we are able to follow gene therapy developments in China more closely than ever before. The majority of the publications that can currently not be retrieved are Ph.D. theses. It is reasonable to expect that in the coming years Western databases will cover the majority of relevant publications from China on gene therapy, including those where some or all of the articles is written in Chinese with an abstract in English provided in most cases.

Data from Chinese literature have been further described in the next chapters on clinical studies and research activities. However, clinical data and shedding data were difficult to find in Chinese literature, most data related to safety issues were retrieved from publications in English and are described in a separate chapter (6) on safety issues. Furthermore, information from Chinese literature is given in chapter 5 especially related to companies and the respective references are listed at the end in chapter 9 References.

3. CLINICAL STUDIES

Overview of clinical trials

The first hemophilia trial and the registrations of Gendicine and Oncorine draw international attention, but other clinical trials have been performed in China as well. The number of trials approved in China (44) appear to be in the same range as the number of trials approved in The Netherlands (48). Figure 3.1 shows the number of trials approved per year in the Netherlands and China, with the number of clinical trials approved worldwide (1443) shown as a reference. The vast majority of the clinical studies in China concern treatment for cancer. This percentage is somewhat higher than as seen for trials worldwide (66.5%, Wiley database in 2007), whereas in The Netherlands has a somewhat lesser focus on cancer compared to worldwide and is also strong in inherited disorders (Schenk et al. 1997). The different trials as listed in Table 3.1 have been obtained from the SFDA database, the Wiley gene therapy clinical database and literature.

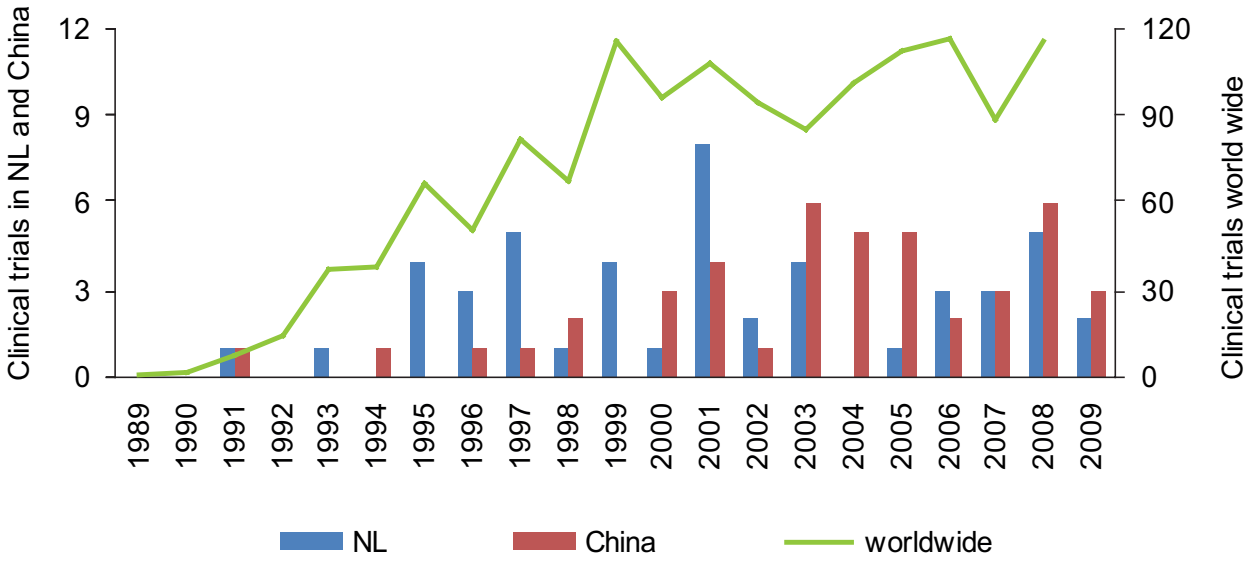


Figure 3.1. The number of clinical trials approved in China, The Netherlands and worldwide (1989-2009). The numbers for the last year, i.e. 2009, for both China and worldwide might be somewhat higher than indicated. The numbers for China are obtained from the SFDA database, the Wiley gene therapy clinical database and literature, the numbers for The Netherlands are retrieved from the VROM vergunningendatabase, numbers worldwide are obtained from the Wiley gene therapy clinical database.

Table 3.1: Clinical studies in China. Data are obtained from the SFDA database (S), the Wiley gene therapy clinical database (CN-#) and literature as listed under source. AAV adeno-associated virus, Ad adenovirus, AIDS acquired immune deficiency disease syndrome, GM-CSF granulocyte monocyte cell stimulating factor, HBV hepatitis B virus, HGF hepatocyte growth factor, HNSCC head and neck squamous cell cancer, HSV-TK herpes simplex virus thymidine kinase, IFN interferon, IL interleukin, RV retrovirus

indication	vector / gene	commencement current status (number of patients)	responsible organization (principal investigator)	source
MONOGENIC DISORDERS				
Hemophilia B	RV (ex vivo) / Factor IX	1991: Phase I (2+2)	Fudan University	Xue, Lu et al 1993, Qiu, Lu et al 1996
Hemophilia B	AAV-2 (ex vivo) / Factor IX	1994: Phase I	Fudan University	Kim, Peng and Kaneda 2008
Hemophilia B	AAV-2 (ex vivo) / Factor IX	2003: Phase I	Fudan University	(S)

CANCER				
Leukemia	RV (ex vivo) / cytokine-activated lymphocyte	1997: Phase I		Kim, Peng and Kaneda 2008
HNSCC	Ad / p53 *Gendicin*	1998:Phase I (190) 2003: Phase II/III (63 / 500 ?) Phase IV (300 ?) 2004: commercial product	Shenzhen Sibiono GeneTech Co., Ltd	(S)
Advanced nasopharyngeal carcinoma	Ad / p53 *Gendicine*	2008: Phase III	Zhang Shanwen Beijing cancer hospital	CN-009
Cervical carcinoma	Ad / p53 *Gendicine*	2008: Phase III	Zhang Shanwen Beijing cancer hospital	CN-010
Advanced thyroid tumors	Ad / p53 *Gendicine*	2009: Phase IV	Jingqiang Zhu Chengdu	CN-013
Advanced thyroid tumors	Ad / p53 *Gendicine*	2009: Phase IV	Longjiang Li Chengdu	CN-014
HNSCC	Oncolytic Ad / ΔE1B [H101] *Oncorine*	2000: Phase I (15) 2005: Phase II (53) Phase III: (66) 2006: commercial product	Shanghai Sunway Biotech Co., Ltd	(S)
Malignant melanoma and non- small lung cancer	Oncolytic Ad / ΔE1B - heat shock protein [H103]	2003: Phase II ongoing		() Li, Liu et al 2009
Advanced solid tumor	Oncolytic Ad / ΔE1B - heat shock protein [H103]	2005 Phase I (27)		Li, Liu et al 2009
Glioma	RV (ex vivo) / HSV-TK pLTKcSN/VPC	1996: Phase I [1996 & 1998 & 2004 in database] Phase I (25) Phase I (14)	Shanghai Neurosurgical Institute and Changzheng Hospital Affiliated Tiantan Hospital, Capital Univ of Med Science	(S) Kim, Peng and Kaneda 2008
Glioma	HSV-I [brainwel]	2002 Phase I completed Phase II ongoing	Royal (Wuxi) Bio-Pharmaceutical Co Ltd	(S)
Glioma	Ad / HSV-TK	2003: Phase I/II		(S) Kim, Peng and Kaneda 2008
Cancer Head and Neck tumor (phase II)	Ad / HSV-TK	2004: Phase I (17) 2007: Phase II (11)	Ma Ding et al Sun Yat-Sen University Cancer Center Shenzhen Tian Da Kang Gene Engineering Co. Ltd.	(S)
Head and Neck and other malignant tumors	Ad / HSV-TK	Phase I (18)	Xu et al State Key Lab of Oncol, Sun Yat-sen Univ, Guangzhou	
Glioma	Ad / HSV-TK	2008: Phase II	Ma Ding Beijing	CN-015
Advanced hepatocellular carcinoma	Ad / HSV-TK	2000: Phase II 2006 closed	Ding Ma Cancer biology Research Center, Wuhan, Hebei	CN-007
Advanced hepatocellular carcinoma	Ad / HSV-TK	2000: Phase II 2005 closed	Beijing Chao Yang Hospital	CN-011

Non-small lung cancer	RV / IL2		N. Mao	CN-002
Gastric cancer	RV (ex vivo) / IL2 modified allogenic gastric cancer cell line vaccine	2001: Phase I (15) Phase II (172?)	Shanghai Second Medical University	(S/2002) Kim, Peng and Kaneda 2008
Cancer	Ad / IL2	2001 2003	Chengdu Hoist	(S)
Gastric cancer	Ad / IL2	2003: Phase I/II		(S)
Cancer	RV (ex vivo) / acivated dendritic cell	2001: Phase I		Kim, Peng and Kaneda 2008
Telomerase positive cancer	Ad / IFN-gamma	Phase I	Q.J. Qian	CN-008
Cancer	Ad / IFN-gamma [E10B]	2006	Huang, Wenlin Zhongshan niersity Guangzhou Doublle BioTech Co Ltd	
Solid tumor	Ad / endostatin [E10A]	2007: Phase II (150 ongoing) 2004: Phase I (15)	Zhongshan University Guanzhou Doublle BioTech Co Ltd	(S)
Head and neck cancer	Ad / endostatin [E10A]	2008: Phase II	Su Yat-sen University	CN-012
Head and neck cancer	Ad / endostatin [E10A]	2008: Phase II	Wenqi Jiang Guanzhou	CN-016
Cancer	Ad / B7-1 + GM-CSF + p53 [BB102]	Pre-clinical	Wu et al Inst of Radiation Medicine, Academy of Military Science	(S)
Solid tumor	Ad / GM-CSF [KH901]	2005 Phase I Phase II Commercial product KH901	Chengdu Kanghong Pharmaceuticals Group	(S) Chang, Zhao et al 2009
Solid tumor	HSV-1 / GM-CSF [OrienX010]	2006 2009 Phase I: ongoing	OrienGen Biotechnol Ltd.	(S)
	HSV-1 / GM-CSF [OrienX010]	2007		(S)
OTHER DISEASES				
Cardiovascular disease	Ad / VEGF	2001: Phase I		(8)
AIDS	Ad / adeno-vaccine + DNA vaccine	2004: Phase I		(9)
Ischemic disease	Ad / endostatin	2004: Phase I		(6)
Ischemic cardiac disease	Ad / HGF hepatocytes	2005: Phase I (18)	First Affiliated Hospital of Nanjing Medical University	(S) (7)
Ischemic limb	Naked DNA / HGF	2008		(S)
Hepatitis B	DNA vaccine / HBV antigen	2005: Phase I		(10)
Hepatitis B	Ad / HBV core antigen (HBcAg)	2005: Pre-clinical	LU Yinying Chinese PLA302 Hospital, Beijing Yu Min Beijing 1st Hospital	(S)

Hemophilia

The first clinical gene therapy trial in China was performed in 1991 delivering a retroviral vector encoding the human factor IX (FIX) gene, to evaluate the therapeutic effect in patients with haemophilia B. This was also the first clinical gene therapy trial for hemophilia worldwide. Even though the results of this clinical trial have been published in Chinese (Qiu et al 1996) and in English (Lu et al 1993) in literature reviews of clinical gene therapy studies performed in China, this particular trial is rarely listed. In reviews of gene therapy for hemophilia, this pioneering work is often only briefly mentioned (eg. Murphy & High, 2008).

Hemophilia is a blood clotting disorder caused by a defective coagulation factor VIII (FVIII; hemophilia A) or FIX (hemophilia B). The first Chinese clinical protocol of human gene transfer for hemophilia B, including points to consider, has been published in Human Gene Therapy (Hsueh, 1992). Using this protocol autologous skin fibroblasts were genetically modified with a retroviral vector containing the human factor IX cDNA (driven by the retroviral LTR promoter) and a double-copy retroviral vector driven by the constitutive cytomegalovirus enhancer/promoter. After a safety assessment, which included: soft-agar test, cell morphology observation, analysis of endotoxin, chromosome karyotype, allergic reaction test, nude mice test, routine pathological test, electromicroscopic analysis, virus detection by PCR, etc., the engineered cells were pooled and embedded in rat collagen mixture. 50 million autologous cells were then injected subcutaneously into the patients' abdomen. Altogether, about 10^9 fibroblasts were injected over the course of 3 or 4 treatments over a period of 3-4 months. Two brothers (aged 9 and 13) suffering from severe hemophilia were treated following this protocol (Hsueh, 1992). The concentration of circulating human FIX protein of patient 1 increased from 71 ng/ml to 220 ng/ml, with a maximum level of 245 ng/ml, where the normal circulating levels of FIX within healthy individuals is 5µg/ml Expression lasted for 6 months at time of writing. The clotting activity increased from 2.9% to 6.3% of normal levels. Patient 2 showed increased circulating levels of factor IX from 130 ng/ml to 220 ng/ml up to 5.5 months at time of writing, with a maximum level of 250 ng/ml. However, the clotting activity was not steadily improved. The expression of factor IX protein persisted in the bloodstream for more than 420 days (Lu et al 1993, Qui et al 1996). The possible reasons advanced for the failure in the older patient were the fact that his fibroblasts grew less well in culture, that he received only 60% of the number of fibroblasts as his younger brother and that he weighed approximately 50% heavier. The presence of the neomycin phosphotransferase gene in the vector could be a problem. Antigen processing of neomycin phosphotransferase within the transduced fibroblasts and their presentation by host-derived major histocompatibility complex class I molecules at the cells surface to specific cytotoxic T cells could be expected in time to result in the clearance of the transduced skin fibroblasts from the treated patients (Brownlee 1995).

While the results were encouraging, the protocol was not repeated with a significantly larger number of patients. Only an additional study with a further two patients was performed and a follow up of the previous first two patients was given where the data obtained were published in Chinese (Qiu et al 1996). An abstract in English, indicated that in the two patients, the values of factor IX protein reached 4-5% of normal levels, while the other two also increased in some extent. 400 days later, one patient was treated again, concentration and bioactivity of clotting factor IX in plasma increased again, and remained thus for more than three years.

Another clinical trial for hemophilia B was approved in February 2003, using a recombinant adeno-associated viral vector (AAV)-2 encoding human factor IX. This trial was performed in collaboration with Beijing Vector Gene Technology Company (VGTC) and the Fudan University (see also chapter 4 for more information on VGTC). No information in English is available.

According to Mannucci the management of hemophilia and allied coagulation disorders has dramatically improved in several Asian countries, due to increased availability of blood products for replacement therapy coupled with the leadership role exerted by a few charismatic physicians, particularly in India and Thailand (Mannucci 2007). In 2007, it was anticipated that China and India both will play an important role in the production of recombinant factors and gene therapy strategies. However, up to now gene therapy for hemophilia has not progressed as expected. Preparations for a new clinical trial for hemophilia are being hampered by practical issues and the trial is not expected to take place in the coming year (oral communication).

Two commercial products for cancer – Gendicine & Oncorine

The next news from the clinic in China that startled the gene therapy field was for the treatment of cancer. On October 16, 2003 and November 4, 2005, the SFDA approved the first two gene-based medicines (Gendicine

and Oncorine resp.) worldwide both for the treatment of head and neck squamous cell cancers (HNSCCs). Gendicine (Shenzhen Sibiono GeneTech) and Advexin (Texas based Introgen Therapeutics), not yet approved by the US FDA, are essentially the same in viral structure. They are a replication-incompetent, recombinant, human adenovirus of serotype 5 virus in which the E1 region is replaced by either Rous sarcoma virus (Gendicine) or a cytomegalovirus (Advexin) promoter linked with the human wild-type p53 tumor suppressor gene and a poly (A) tail. The p53 gene is one of the most important tumor suppressor genes present in normal cells and has also been called the 'guardian of the genome'. The p53 gene is mutated or deleted in approximately 50-70% of human tumors. Introduction of exogenous wild-type p53 gene and subsequent overexpression of the p53 protein has been shown to control and eliminate tumor cell growth by cell cycle arrest or apoptosis. Furthermore, the overexpression of p53 protein has been demonstrated to have a synergistic effect with radiotherapy and chemotherapy.

Deletion of the E1A region prevents Gendicine from replicating, a feature introduced for safety reasons, since infectivity is limited to only one cycle. However, this modification could hamper efficacy. In contrast, oncolytic viruses are viruses that are able to replicate specifically in and destroy tumor cells, a property that is either inherent or genetically-engineered. Oncorine (H-101), the second approved gene-based medicine, is a recombinant oncolytic adenovirus that selectively replicates in cancer cells deficient in the tumor suppressor gene p53 and destroys them while sparing normal cells. H101 has a E1B-55kDa deletion and a partial E3 deletion. H101 is essentially the same as Onyx-015 (dl1520 or CI-1042), that was previously developed by Onyx Pharmaceuticals. Only H101 has a slightly larger deletion in the E3 gene, which affects the immune response.

Gendicine

Gendicine has currently been approved only for head and neck cancer with intratumoral injection and in combination with chemotherapy. A patient receives one injection of Gendicine per week for four to eight weeks consecutively as a treatment cycle. A standard dose of Gendicine is 1x10E12 viral particles. A number of clinical studies have been performed with an off-labeled use of Gendicine which is allowed with permission of individual institutional ethical committees. The off-labeled use of Gendicine has been tested for lung cancer, breast cancer, mesothelioma, and esophageal, hepatocellular, gastric, colorectal, and pancreatic carcinomas. In off-label use various administration routes have been given, including pleural and peritoneal cavity perfusion and even intra-arterial as well as intravenous administration.

Clinical results indicated that Gendicine shows no serious adverse effects. Some patients experienced self-limiting Grade I and II fevers lasting approximately three hours in general. Results were presented by Dr. Peng, founder of Shenzhen Sibiono GeneTech, at the European Society of Gene and Cell Therapy meeting held in Rotterdam, October 2007. At that time more than 7000 cancer patients, including 500 non-residents had received Gendicine. Individual case studies presented were impressive, however, convincing data of studies with larger number of patients with clear selection criteria following a strict treatment schedule were being missed.

Phase II and phase III data for Gendicine were obtained from 135 patients (Peng 2005), far fewer than the number of patients required for phase III trials conducted in the USA or Europe. In February 2009, results from a 6 year follow up study of Gendicine were published in the Journal of Clinical Oncology. In the randomized, controlled clinical trial in patients with nasopharyngeal carcinoma (NPC), Gendicine was combined with radiotherapy and compared to treatment with radiotherapy alone. Patients with NPC receiving combination therapy (n=42) showed a 2.73 fold higher complete response rate (66.7% versus 24.4%) than patients only treated with radiotherapy (n=40). The 5 year local regional tumor control rate was significantly increased by 25.3% in patients receiving Gendicine in combination with radiotherapy as compared to patients receiving only radiotherapy. No adverse events or dose limiting toxicity were observed during the 6 year follow up study, except for transient fever after Gendicine administration. However, the differences in survival observed were not statistically significant. The 5 year overall and 5 year disease free survival rate of patients receiving Gendicine combined with radiotherapy were increase by 7.5 (p= 0.34) and 11.7% (p=0.21), respectively, as compared to patients receiving radiotherapy alone. A trial with a larger number of patients is planned in order to see whether this could render statistical significance.

Additional trials with Gendicine were initiated in April 2008 (phase III trials of Gendicine combined with radiotherapy for the treatment of NPC and cervical cancer) and in 2009 (phase IV trials of Gendicine for the treatment of HNSCC, advanced oral and maxillofacial malignant tumors, and advanced malignant thyroid tumors). Furthermore, results were published from studies of patients with dysplastic oral leukoplakia, gastric

cancer, colon cancer and esophageal cancer treated with Gendicine showing biological activity [3 articles in Chinese]. In addition, a pilot phase II trial of multiple hepatic arterial injections of Genedince and 5-fluoruracil (5-FU) after transcatheter arterial chemoembolization (TACE) of unresectable hepatocellular carcinoma was reported. Overall survival times were 12.8 months (range 2.7-26.2 months) in patients receiving multiple hepatic arterial injections of Gendicine and 5-FU after TACE (n=23) and 10.4 months (range 2.7-22.5 months) in patients being treated by TACE alone (n=23).

From 5 Dutch patients receiving Gendicine treatment very limited information is given at the end of chapter 7. The total claimed number of 50.000 patients treated with Gendicine exceeded the number of patients in clinical trials in Western countries. The further increase of results obtained from Gendicine treatment could be informative for cancer gene therapy studies in general.

Oncorine

Phase I and phase II clinical trials with Oncorine included 191 patients with 13 kinds of carcinoma such as head and neck cancer, breast cancer, and colon cancer, phase III clinical trials included patients with head and neck cancer. Oncorine was injected intratumorally at the dose of 5x10E11 vp for 5 consecutive days or a treatment course. The adverse effects were fever, local pain tat the injection site and flu-like symptoms, not serious and well tolerated (Ma et al 2008).

From 2000 to 2004, patients (n=228) with advanced cancers were recruited for phase I to phase III clinical trials of H101 (Oncorine) in China. Both naïve patients and patients that were refractory to chemotherapy were studied. The percentage of patients that experienced an objective clinical response (either partial or full tumor shrinkage) was increased in the cohort receiving the combination of H101 and chemotherapy compared with the group receiving chemotherapy alone (73 versus 40%) (Yu et al 2007/Shi & Zheng 2009).

Interestingly, the patients who had fever at the time of Oncorine administration had better objective responses than those without fever although statistically not significant. The precise mechanism remains uncharacterized but preclinical data suggest that it was attributable to elevated expression of the heat shock protein, which facilitated late viral RNA export to cytoplasm ad subsequently enhanced viral replications.

Another study of trimodal combination of Oncorine, chemotherapy, and radiotherapy was conducted for 56 patients with nasopharyngeal carcinoma (Lu et al 2006). Although clinical results of the control group without Oncorine administration were not shown, the trimodal therapy showed 71.4% of complete responses and 28.6% of partial responses with minimal adverse effects such as transient fever and mucosal ulcer in the oral cavity.

Additional clinical trials for cancer

Conditionally replicating adenoviral vectors

In clinical trials using H101, it was noticed that the patients with fever after H101 intratumoral injection tended to show higher tumor regression response rates, not only in the locally injected tumor but also in the uninjected distant tumor masses, than the treated patients without fever. One of the best-characterized biological responses to fever is the induction of heat shock protein (HSP) expression. HSPs have the ability to chaperone and present a broad repertoire of tumor antigens to dendritic cells, and activate both innate and adaptive antitumor immune responses and have been extensively tested in clinical trials. Based on these observations, Shanghai Sunway Biotech developed H103, another conditionally replicating adenovirus with HSP70 gene using a CMV enhancer (Li et al, 2009). In preclinical studies in mice, cavies or rhesus monkeys local administration of H103 showed no significant toxicity. Intratumorally injection of H103 in a dose-escalation study (2.5x10e7 to 3.0x10E12 viral particles, VP) was given to a total of 27 patients with advanced solid tumor. Dose limiting toxicities were observed in 2 patients at the dose of 1.5x10E12 and 3.0x10E12 VP, grade III fever and transient grade IV thrombocytopenia resp. The maximum tolerated dose of H103 was not defined. The adverse events were mainly mild to moderate (grade I/II) in nature, including fever, mild injection-site reaction, leucopenia, lymphopenia, thrombocytopenia and hypochromia. The objective response (complete response+partial response) to H103-injected tumors was 11.1% (3/27), while the clinical benefit rate (complete response+partial response+minor response+stable disease) was 48.1%. Interestingly, in three patients transient and partial regression of distant, uninjected tumors was observed. A next phase II clinical trial of H103 is currently ongoing with patients with malignant melanoma and non small cell lung cancer (Li, Liu et al 2009).

Tumor vasculature targeted therapy

A lot of attention has been given to the clinical studies initiated by dr. Peng of Shenzhen Sibiono GeneTech and dr. Hu of Shanghai Sunway Biotech. A third player in the cancer gene therapy field in China is Wenlin Huang,

professor at the Sun Yat-sen University and founder of Guangzhou Doublle Bioproducts.

This group is working on tumor vasculature targeted therapy. Endostatin (E10A) is an angiogenesis inhibitor that has been widely used and this gene was inserted into an adenoviral vector.

Previous studies showed that endostatin gene delivered by adenoviral vector significantly inhibited the growth of BEL-7402 in xenograft liver tumors model. On day 24 after intratumor injection with Ad-rhE, the T:C ratio of mean tumor volume was less than 50%, and the growths of xenografted tumors were significantly inhibited as compared with the control group after six courses of treatment of Ad-rhE (Li et al., 2004). Our another study showed multiple injections with adenoviral vectors did not lead to continuous increase in serum neutralizing antibodies against the adenovirus, adenovirus-mediated intra-tumoral introduction of human endostatin gene may form a viable treatment for NPC (nasopharyngeal carcinoma), readministration every 2–3 weeks may be necessary for best effect (Li et al., unpublished data).

In conclusion

Currently, it is difficult to directly compare clinical gene therapy performed in China and outside China. It has been suggested that Chinese studies showed better therapeutic effects with the same adenoviral vectors. Reasons for this would be the differential eligibility criteria, Chinese studies sometimes included early staged patients who should be treated with a standard conventional therapy. Secondly, Chinese studies often use the gene therapy treatment, esp. Gendicine, as an adjuvant to the standard therapy (Ma et al 2008), and the standard therapy use as control arm in the Chinese studies are usually not the standard therapy outside China.

4. RESEARCH ACTIVITIES

Developments over time – disease areas and vector technologies

The CNKI database was further used for analysing the developments over three time periods: 1996-2000, 2001-2005 and 2006-2010. Separate focus was on different target diseases using gene therapy approaches as well as on different gene transfer technologies. Major disease area investigated is cancer (starting from 40% in the first period up to 50% in the last period), which is also the main focus in the rest of the world. Next most studied diseases (from first to last period in relative percentages) are liver related disorders (from 6% up to 11%), neurological disorders (from 7% up to 11%), heart and cardiovascular diseases (from 3% up to 6%), diabetes (1-2%). More details are shown in Table 4.1.

Table 4.1 Trends over time – disease areas. Gene therapy publications as found in the CNKI database, showing distribution of publications related to different target diseases.

disease	1996-2000	2001-2005	2006-2010
Cancer	39,4	39,2	50,7
Liver related	5,9	9,4	11,2
Neurological	5,1	7,5	9,3
Parkinson	2,0	1,5	1,0
Alzheimer	0,2	0,2	0,3
Ocular	0,4	0,8	1,6
Heart	1,0	2,3	3,6
Cardiovascular	2,1	1,9	1,9
Ischemic	1,4	3,7	3,7
Hemophilia	1,7	0,9	0,6
Genetic	2,3	1,8	1,8
Lung	0,7	1,0	1,3
Arthritis	0,3	1,0	0,6
Diabetes	1,1	1,8	1,9
Other	35,1	26,1	9,5

Table 4.2 shows the trends over time for gene transfer technologies. Traditionally, Chinese scientists publish a lot of reviews, in these vector technology searches reviews are accounting for 14% to 19% of the total number of publications. Non-viral approaches are described in 19% up to 43% of the studies published when comparing publications from 1996-2000 with most recent five years. Viral vector mediated gene transfer studies show a small decrease from 31% to 29%. The adenoviral vectors are still popular and show an increase from 12% to 17%. Retroviral vectors are studied significantly less (14% to 4%), this decline most likely favours the use of lentiviral vectors (0.2%-2.8%). Herpes simplex virus (2.7-1.6%), poxvirus (0.1%), Sendai virus (0.1%), vaccinia virus (0.3-0.1%) is studied only limited.

Table 4.2. Trends over time – vector technologies. Gene therapy publications as found in the CNKI database, analyses related to different gene transfer technologies.

vector	1996-2000	2001-2005	2006-2010
RNA*	6,5	12,8	23,7
Antisense (excl RNA*)	1,9	1,1	0,5
Lipofection	9,5	14,7	17,7
Adenovirus	12,1	15,8	16,7
AAV	1,3	2,5	3,7
Retrovirus	14,1	7,0	3,5
Lentivirus	0,2	0,4	2,8
Herpes simplex virus	2,7	1,9	1,6
Poxvirus	0,0	0,1	0,1
Sendai virus	0,0	0,1	0,1
Vaccinia virus	0,3	0,1	0,1
Hepatitis virus	0,0	0,0	0,0
Measles virus	0,0	0,0	0,0
DNA vaccine	0,5	0,7	0,8
Naked DNA	0,3	0,6	0,5
General reviews	14,0	16,3	18,6
Other	36,6	25,8	9,6

The status of gene therapy in China

Dr. Xin Yuan Liu, member of the Chinese Academy of Sciences, is the Chinese scientist that has published the most in the cancer gene therapy field. He was also one of the speakers of the Gene Therapy Seminar held last June 14 on the occasion of the Dutch Life Sciences Week in Shanghai. During his presentation he stressed that gene therapy is just one of the treatment options for cancer and that we should realise the importance of the other optional treatments eg. growth factors. His message on putting gene therapy in perspective has also been send across by many Chinese scientists, both inside as outside the gene therapy field. Gene therapy is not being regarded as the solution to many medical urgent needs. This is an important signal, since Western audience might misconceive the previous promotion of Gendicine and the energy put into the promotion of gene therapeutic medicines in China. In general, scientists believe that there are still many hurdles to take before gene therapy will be put into clinical practice. Below is given a short overview of various preclinical gene therapy studies and a more extensive overview is given of haemophilia as an example of ongoing activities.

Current preclinical studies

Relatively new are the lentiviral studies. A mouse model of established large-sized hepatocellular carcinoma was

used for intratumoral injection of lentivirus-shRNA (short hairpin RNA) or siRNA targeting EZH2 (enhancer of zeste homolog 2) resulted in significant tumor regression (Chen et al 2007).

Several investigators in China are working on RNA-based gene therapy, particular using siRNAs (Zheng et al 2009, Shi et al 2009, He et al 2008). In addition, various types of nanoparticles are being developed for possible use as gene delivery tools (Yu et al 2008, Zhang et al 2009).

Furthermore, new oncolytic viruses are being developed, including fiber-modification. SG235 with both and E1B 55-kDa deletion and an Ad5/F35 chimeric fiber, for the treatment of B-cell tumors (Wang et al 2009). SG235 expressing exogenous TRAIL induced the apoptosis of leukemic cells and elicited an anti-leukemia activity as compared with SG235 (Jin et al 2009).

Hemophilia

The clinical studies on hemophilia are the work of one group at the Fudan University, Shanghai, in collaboration with Vector Gene Technology Company, Beijing. Preclinical research for Hemophilia is performed in several different groups around China using different type of strategies. An overview is given below. In 2010, the group of Dr. Zhugang Wang at the Shanghai Research Center for Biomodel Organism published a paper (in Chinese, abstract in English) on the generation of a factor VIII gene knockout mouse by tetraploid embryo complementation technology. However, already in 1995 a hemophilia A mouse model, using gene targeting resulting in severe factor VIII deficiency, have been described (Bi et al 1995). Other recent papers (in Chinese, abstract in English) are on various delivery vectors (LV, AAV, Ad, non-viral) for Hemophilia A or B. A paper written by from Lü *et al* 2009 (from the Institute of Molecular Medicine, Fujian) reviews the preparation, expression and lion of self-complementary AAV vectors both *in vitro and in vivo*, with a specific focus on recent progress of scAAV-based vectors to treat hemophilia B. A research article demonstrated a dose-dependent therapeutic effect of rAAV2-hFIX injected into muscles of the hemophilia B mouse model. Peak levels of hFIX expression were found at three weeks after injection whereafter levels gradually decreased and expression was still detectable upto 10 weeks after injection. No rAAV2 vector DNA was detected in other organs other than the injected muscle tissue (Peng *et al* 2004, Department of Hematology, Xiangya Hospital of Central South University, Changsha).

Chen and colleagues described the expression of human factor IX from retrovirus-transduced human umbilical cord tissue derived mesenchymal stem cells (Chen *et al* 2009). Previously, in 2004, Kang and co-workers described *ex vivo* transduction of retrovirus transduced bone marrow stroma cells. However the development of anti-FVIII antibodies in recipient mice in the first week after injection hampered expression. In a subsequent study, the recombinant retroviral vector encoding the human FVIII cDNA was administered intravenously via the X vein. Expression of hFVIII int plasma to physiological levels were measured 48 hrs after injection (average hFVIII was 0.62 U/ml; peak 0.89 U/ml; n=6) although circulating levels of hFVIII gradually decreased over time.

Another study described the use of intestinal epithelial cells transfected with plasmid vector containing the human factor IX gene. This resulted in a peak of expression after 48 hr, decreasing by 50% within 72 hr (Du *et al* 2008; Department of Hematology, Henan Tumor Hospital, Zhengzhou).

A more challenging non-viral approach was performed by using a human ribosomal DNA-targeting vector. Site-specific integration was observed with an efficiency of 1.1x10E-5. Expression could subsequently be detected (4.3 +/- 0.9 ng 10E6 cells at 24 hr) (Liu et al, National Laboratory of Medical Genetics of China, Changsha in J Throm Haemost).

Furthermore, several research papers are describing the use of lentiviral vectors. The department of Hematology of the Affiliated Hospital of Xuzhou Medical College in Xuzhou reported on the expression of recombinant canine factor VIII *in vitro*. Lentiviral vector titers reached up to 1.54x10E6 U/ml and 2.83x10E6 U/ml. Activity of cFVIII could be detected at 24 hrs after infection of mammalian 293T cells and reached highest levels at 72 hrs. This was close to canine FVIII activity of normal dog plasma. Six weeks later, the expression level was ¼ of the highest level measured. In a following paper, the human FVIII gene was used in a lentiviral vector for infection of 293T cells and similar results were obtained. Already in 2004 the group of Dr. Xue (State Key Laboratory of Genetic Engineering, Fudan University, Shanghai) published a paper in English regarding the delivery of hFIX after injection of lentiviral vectors *in utero*. The highest level of hFIX was 50 ng/ml and lasted for more than 30 days. Expression was only detected in the liver. hFIX cDNA was detected in the liver, spleen, and heart and no germ line transmission was observed. In addition, this same group also reported on a study of gene transfer and expression of hFIX in hemophilia mice mediated by a mini-adenoviral vector (Gao et al 2003).

5. STATE PROGRAMS, INDUSTRIAL ACTIVITIES AND INTERNATIONAL COLLABORATIONS

State programs

The modern Chinese biotechnology industry started in the eighties, after the first waves of reform in China. The Key Technologies Research and Development Program started in 1982 and is considered the largest scientific and technological plan in China in the 20th century. This program involves tens of thousands of people in more than 1,000 scientific research institutions nationwide. The program aims at a comprehensive approach to the key problems directing the national economic and social development, covering agriculture, electronics, informatics, energy resources, transportation, materials, resource exploration, environmental protection, medical and health care, among other fields.

In March 1986, the Hi-Tech Research and Development Program (Program 863) was issued after extensive and strict appraisal by hundreds of Chinese scientists. The program has established 20 subjects covering 8 fields, including biology, aerospace, informatics, laser, automation, energy resources, new materials and oceanology. For the gene therapy field the most relevant objective is to develop bioengineering technologies 'to improve the quality of life'. Program 863 is noteworthy for the rapid industrialization of its research findings and international cooperation.

In June 1997, The National Program for Priority Basic Research and Development (Program 973) was approved, with a focus on the application of basic sciences. Topics are the major scientific issues of agriculture, energy resources, informatics, resources environment, population and health care, and materials. More specifically, it includes Applied Basic Research on Gene therapy and Stem Cell Research: Basic Research and Clinical applications and Scientific Research on Fundamental Issues of Tissue Engineering.

Furthermore, in 1993 China first allowed for the patenting of medicines, a major milestone for its pharmaceutical industry. In the late nineties several bioethical regulations were issued by the authorities (Doering, 2003) and in 2001 China joined the World Trade Organization indicating the transformation towards a reliable legal state.

China has been encouraging the return of Chinese scientists and entrepreneurs who left the country to study or work abroad, and to turn the 'brain drain' into a 'brain gain'. The highly qualified personnel that returned, also known as 'sea turtles', bring scientific talent as well as international credibility (Frew et al, 2008).

In a comment on the successes of Shenzen Sibiono GeneTech and Shanghai Sunway Biotech to get approval of the first and so far only gene therapy medicines in the world (as listed in Table 5.1), Zailin Yu (president of Beijing-based drug development company Bioway–Fortune Gene Technology Research Center) says that this is the result of heavy, even desperate, government investment. The problem according to Yu is that "China's venture capitalists, pharmaceutical firms and the stock market are not ready to support an overall 'great leap forward' in the biotech industry" (Jia & Kling, Nat Biotechnol 24, 2, 2006).

Table 5.1: Gene therapy medicines that obtained approval
(Jia & Kling, Nat Biotechnol 24, 2, 2006)

Approval date: October 16, 2003
Gendicine (recombinant adenovirus type 5 carrying human p53 gene)
Approved for head and neck squamous cell carcinoma
henzhen Sibiono GeneTech (Shenzhen)
This is the first commercialized gene therapy product worldwide (Nat Biotechnol 22, 3-4, 2004). Currently, Texas-based Introgen Therapeutics is waiting for the FDA's approval for its p53 gene-based Advexin, licensed from the M.D. Anderson Cancer Centre in 1993. This drug is similar to Gendicine.
Approval date: November 4, 2005
H101 (recombinant oncolytic adenovirus type 5)
Approved for head-and-neck squamous-cell carcinoma
Shanghai Sunway Biotech (Shanghai)
H101 selectively replicates in cancer cells deficient in the tumor suppressor gene p53 and destroys them while sparing normal cells. H101 is essentially a modified version of Onyx-015, initially developed by Onyx Pharmaceuticals, and later abandoned since Onyx chose to focuses on kinase inhibitor Nexavar (sorafenib), approved in late 2005 (Nature Biotechnol 2, 1453-1454, 2005).

Companies

From the mid-nineties on, several companies with an interest in gene therapy and genetic vaccines have been founded in China. These are mainly based in Beijing, Shanghai, Shenzhen and Chengdu. An overview is shown in Figure 5.1 and Table 5.2.

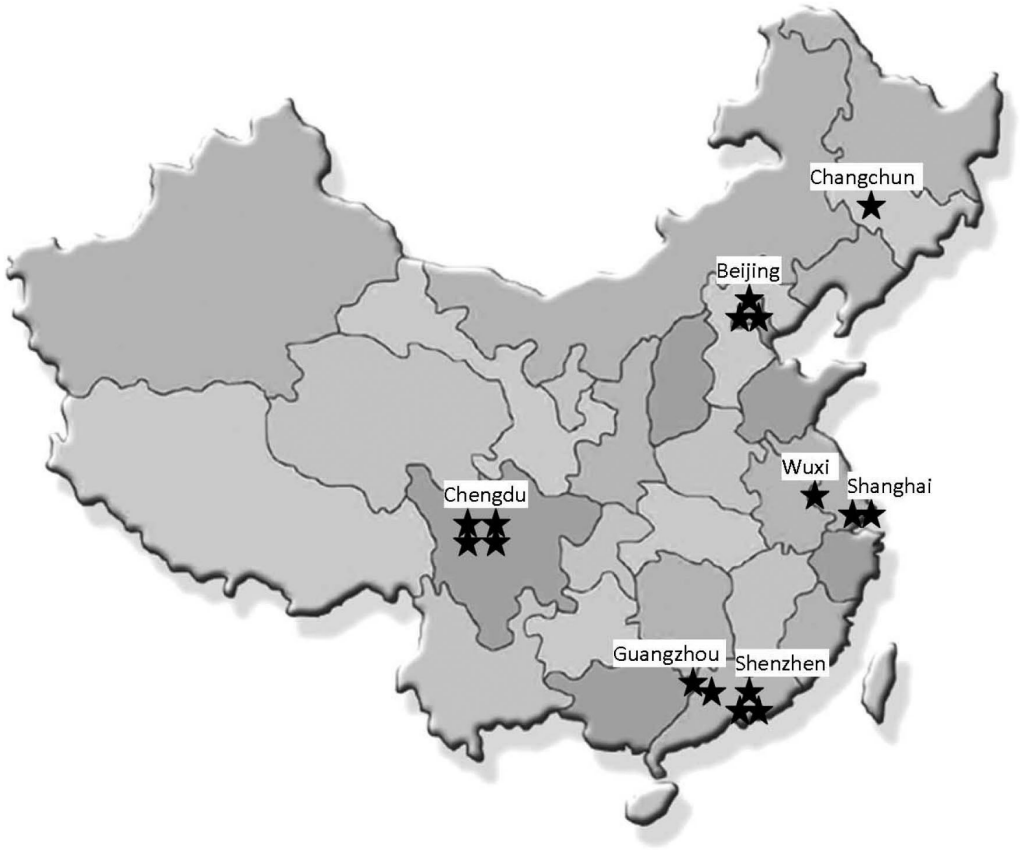


Figure 5.1: Companies with an interest in gene therapy are located in major cities in China.

Table 5.2: Chinese companies devoted to gene therapy or genetic vaccines

Company	Product
Shanghai Sunway Biotech co. Ltd. (Shanghai)	Oncorine (H101) / head and neck squamous cell carcinoma, H102, H103
Shenzhen SiBiono GeneTech (Shenzhen)	Gendicine (Ad-p53) / head and neck squamous cell carcinoma
Beijing Vector Gene Technology Company (Beijing)	AAV, RV, HSV systems AAV/Hemophilia
Shenzhen Tian Da Kang Gene Engineering Co. Ltd. (Shenzhen)	Ad-HSV-TK
Royal (Wuxi) Bio-Pharmaceutical Co. Ltd. (Chengdu)	'brainwel' HSV-TK
Chengdu Hoist Group Co. Ltd (Chengdu)	Ad-IL-2
Guangzhou Doublle BioTech Co. Ltd. (Guangzhou)	Ad-rhE, Ad-rhIFNγ
Chengdu Kanghong Pharmaceuticals Group (Chengdu)	KH901 (Ad-GM-CSF)
OrienGen Biotechnology Ltd. (Beijing)	OrienX010 (HSV-1-hGM-CSF)
Telebio Lentiviral Vector Research Center (Shanghai)	Lentiviral vector production services
Chongqing Zongsheng Biotech (Chengdu)	Tiefu (Ad-CTLA4Ig expressing in pig skin cells)
[acclaimed not to belong to gene therapy since not modifying human tissue]	
	Genetic vaccines (clinical phase)
Changchun Baike Pharmaceutical Inc Co., Ltd. (Jilin)	AIDS vaccine (Phase I: 2004, Phase II: 2007)
Beijing Tiantan Biological Products Co., Ltd. (Beijing)	AIDS vaccine, using poxvirus
Guangzhou Baidi Biotechnology Co., Ltd (Guangzhou)	Hepatitis B vaccine, double plasmid
Shenzhen Tsinghua Yuanxing Pharmaceutical co., Ltd. (Shenzhen)	Ad-HB vaccine
Shanghai Fudan Yueda Biotech co., Ltd. (Shanghai)	Hepatitis B [HHsag-HHig(ic)] vaccine

Shanghai Sunway Biotech Co. Ltd.

Shanghai Sunway Biotech Co. Ltd. was founded in December 1995 as a sub-company of Shanghai Sunve Pharmaceuticals Co., Ltd. Later, Mergen Biotech Ltd. became a shareholder and since 2003 holds 100% of the shares. On November 4, 2005, Oncorine recombinant human adenovirus type 5 injection (H101), developed by Shanghai Sunway Biotech, became the second gene therapy medicine approved by Chinese SFDA.

Shanghai Sunway Biotech develops, manufactures and commercializes cancer biotherapeutics based on its oncolytic viral technology. The commercial gene therapy product Oncorine is a genetically engineered oncolytic adenovirus with an E1B deletion and a partial E3 deletion. It targets tumor cells carrying a dysfunctional p53 gene and results in cytolysis of these cells. Oncorine is currently in its phase III clinical trials. Besides Oncorine (H101), Shanghai Sunway Biotech develops a tumor-targeted recombinant adenovirus injection (H102) and an oncolytic recombinant adenovirus injection (H103) for treatment of cancer. H102 specifically targets primary hepatocellular carcinoma and replicates itself to destroy the tumor cells. H103 adenoviruses lyses the tumor cells and expresses Hsp70 that can potently stimulate an anti-tumor immune response. H102 is still in the preclinical stage, while H103 has finished its phase I clinical trial.

Eleven international patent applications have been filed (including an exclusive worldwide patent for the E1B-B55KD adenovirus), with six issued; ten patent applications have been filed in China with one issued. Shanghai Sunway Biotech holds an exclusive worldwide license for Onyx Pharmaceuticals' (Emeryville, CA, USA) Onyx-015 oncolytic adenovirus against head-and-neck cancer (Frew et al 2008).

Shanghai Sunway Biotech has collaborations with Sun Yat-Sen University Cancer Center (Guangzhou, China) to run phase I-III clinical trials of H101 for head-and neck squamous cell carcinoma and with Tumor Hospital of Medical Sciences Academy of China (Beijing) to run a phase I clinical trial of H103 in cancer. In addition, they work together with Peking Union Medical College Hospital (Beijing), Zhongshan Hospital of Fudan University (Shanghai) and Renji Hospital of Shanghai Jiaotong University (Shanghai) for developing a gene therapy treatment of peripheral artery disease (Frew et al 2008). Shanghai Sunway Biotech ran a pilot production for Genzyme (see under Genzyme), however there has been no follow up so far.

Shanghai Sunway Biotech Co. Ltd. had up to 120 employees working in R&D, but recent cuts in R&D have shrunk the workforce to only 40 employees. The main focus of the company is now on recombinant human granulocyte colony-stimulating factor injection (rhGCSF, brand name SunGran®). SunGran sales are currently the major source of income for the company.

Shenzhen Sibiono GeneTech (Shenzhen)

In March 1998, the Shenzhen Sibiono GeneTech company was founded by Dr. Zhaohui Peng. Nearly all of its RMB 80 ($9.6) million research funding was received from government bodies, public foundations and major universities (Jia & Kling, 2006). The clinical trials for Gendicine were launched later that year. In 2000, the company established its gene therapy GMP production line and GLP lab.

Shenzhen Sibiono GeneTech also participated in drafting the "Points to Consider for Human Gene Therapy and Product Quality Control" which was published by SFDA in March 2003 and has become the national technological legal document.

On October 16, 2003, Shenzhen Sibiono GeneTech obtained the Drug license, Production Approval and GMP Certificate from the China State Food & Drug Administration (SFDA) for Gendicine. Subsequently, on February 13, 2004 the Shenzhen Sibiono GeneTech facility passed GMP approval by SFDA. The released final product has the following quality characteristics: IU/VP ratio of bout 4.8%, purity over 97%, and less than 1 RC in 3x10E10 VP.

The company's website states that Shenzhen SiBiono GeneTech developed two major technical platforms: the Viral Vector Gene Delivery System and Non-Viral Vector Gene Delivery System, and currently focuses its research and development on commercializing and developing gene therapy products for treating cancerous, cardiovascular and infectious diseases.

Four international patents were filed through PCT for Gendicine, along with seven more patents filed in China. Of those, product invention and manufacture technology patents have been issued in China; the other five are pending (Frew et al 2008). According to Chinese sources, Shenzhen SiBiono GeneTech is also reportedly holding negotiations with its US counterpart Introgen Therapeutics, based in Austin, Texas to enter the US market (Jia & Kling, 2006).

However, the successful growth of SiBiono is also accompanied by controversies, which started with discussions regarding the efficacy and safety of Gendicine. The available clinical data and conclusions drawn were obtained from a relatively small number of patients in

clinical trials (n=12 in phase I and n=135 in Phase II/III, Peng review), much smaller than required in the USA or Europe (Juan Shi review). Although a much larger number of patients (more than 2500) have been treated and inspected in these clinical trials (Peng review), and probably an even larger number of patients are actually using the medication, the clinical data of these patients are hard to access, partly due to the fact that they were published only in Chinese journals. Furthermore, the effectiveness of the medication, e.g., in survival, does not significantly exceed that of other treatment, e.g. radiotherapy, as shown in the latest publication of Gendicine clinical study (Pan JJ, 2009).

SiBiono was short of resources to expand its clinical trials and production and was acquired in 2006 by USA-listed Chinese pharmaceutical firm Benda Pharmaceutical (Hubei, China), through its 95% owned China-based subsidiary Hubei tongji Benda Ebei Pharmacuetical (Hubei, china), owning a 57.6% majority share. Conflicts emerged between the major shareholder Benda and the head scientist Dr. Zhaohui Peng after only a few months of collaboration, which later led to the resignation of Peng from SiBiono in 2008 and a subsequent lawsuit to fight for the patent of Gendicine that continues today (Jia, 2009). Presently, the patent is still in Peng's name, at least until the ruling of the lawsuit is made. Meanwhile, Benda itself is drowning in another lawsuit in the United States and requests to be liquidated by its takeover companies. The future of Shenzhen SiBiono GeneTech and Gendicine is therefore unclear.

Beijing Vector Gene Technology Company

Beijing Vector Gene Technology Co., Ltd. (VGTC) was founded in October 2000 as a biotech company focusing on research and development of viral vectors. Since 2002 the company has established a GMP facility, GLP laboratory and SPF animal laboratory, and became a professional provider of pre-clinical and clinical grade gene vectors used for gene therapeutics and gene transfer research, e.g. adeno-associated virus, adenovirus and plasmid DNA. Since August 2005, VGTC was invested by the state-owned Beijing Pharmaceutical Group Co., Ltd. to stimulate the development of VGTC. Currently, the company is specialized in large-scale manufacturing of AAV vectors and developing novel viral vectors. It is recognized as the R&D Base of Viral Gene Vector under the National Hi-tech Research and Development Program of China 863 Project, and is authorized by the Ministry of Personnel of China to host post-doctoral researchers.

In February 2003, the first clinical trial of gene therapy using recombinant rAAV2 to express factor IX in hemophilia B patients was approved by SFDA in China. This gene therapy clinical trial on 4 hemophilia B patients was operated by VGTC and Dr. Jinglun Xue's research group in Fudan University, Shanghai, China. To date, the clinical trial of rAAV-FIX has concluded its phase I stage. VGTC is also involved in preclinical studies of an oncolytic recombinant AAV injection SG600-P53 for treating malignant tumors, and a recombinant AAV2 anti-tumor necrosis factor injection rAAV2/TNFR:Fc for treating rheumatoid arthritis (Chinese Journal of Virology 21(3): 204-209, 2005. Gao, Kai et al.). These two vectors are waiting for the SFDA approval to start their clinical trails.

Shenzhen Tian Da Kang

Shenzhen Tian Da Kang Gene Engineering Co., Ltd. (Tian Da Kang) was founded in 2001 in Shenzhen as a private returned-student enterprise. The company aims at research, development, production and clinical trials of genetic engineering medicines.

The current project of Tian Da Kang is the recombinant Adenovirus - Herpes Simplex Virus Thymidine Kinase (shortened as ADV-TK). The Ad-TK construct was made in the research group of Prof. Ma Ding, Cancer Biology Research Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. It was approved by SFDA to enter phase I clinical trials in July 2004 and phase II clinical trials in 2007. ADV-TK may be effective in treating various cancers including liver cancer, lung cancer, breast cancer, gastric cancer, ovarian cancer and prostate cancer. The completed phase I clinical trial was conducted on 17 patients of various types of tumor at Sun Yat-Sen University Cancer Center. Preliminary medical effects can be seen from the 17 evaluable cases (efficacy rate of 38% with ADV-TK on a single-use basis), according to the company's website information (http://www.ship.gov.cn/index.asp?bianhao=9598). A publication of the ADV-TK phase II clinical trial in 2007 reported that ADV-TK was used as an adjuvant treatment for patients (n=45) with hepatocellular carcinoma (HCC) who underwent liver transplantation. The additional ADV-TK treatment resulted in significantly higher recurrence-free and overall survival than liver transplantation alone (Li N et al. Clin Cancer Res 2007 13(19): 5847-5854). A phase II clinical trial of using ADV-TK treating head and neck cancer patients (n=11) is currently ongoing (Ren Guoxin et al., Eighth National Oral and Maxillofacial - Head and Neck Tumor Conference Papers Series 2009, in Chinese).

A phase II trial of ADV-TK for the treatment of patients with gliomas is ongoing (NCT00870181, according to review Current Opinion in Molecular Therapeutics 2009 11(5): 547-553).

Royal (Wuxi) Bio-Pharmaceutical Co. Ltd

Royal (Wuxi) Bio-pharmaceutical. Co., Ltd., is a joint venture of Chinese and foreign investments. Its sister(?) company in Vancouver, Canada, was founded in 2000 and is the resource for the core technology for Royal (Wuxi), according to the company's website information (www.chinaroyal.cn). Royal (Wuxi) has GMP production factory, research and animal facilities and covers over 100,000 m^2. It mainly works on the research, development, production and commercialization of biomedicines, including genetic engineering products, diagnostics and preventive biological products. The scientific leader of the company's Canadian technological team is Sr. William Weiguo Jia, a renowned professor in the field of oncolytic virotherapy.

Royal (Wuxi) is currently developing oncolytic gene therapy products to treat cancer. Brainwel, a genetically modified type I herpes simplex virus (HSV-I) carrying angiostatin and endostatin fusion genes, is the first oncolytic HSV-I entering clinical trials in China. It was approved by SFDA in October 2002 for its phase I clinical trial to treat human malignant brain glioma and is now in its Phase II stage. For other genetically modified type I herpes simplex virus products for treating liver cancer and lung cancer, preclinical research has either been completed or is still ongoing.

Chengdu Hoist Group Co. Ltd

Chengdu Huasun Group Inc. Ltd. is mainly engaged in the manufacture and distribution of Chinese medicines and chemical drugs, as well as steel structures and their design and property distribution. It is a Shenzhen Stock Market listed company since March 1998. Chengdu Huasun Biotechnology Co. Ltd., (Chengdu Hoist) is a subsidiary of the Chengdu Huasun Group Inc. Ltd.. It is an established high-tech enterprise, specialized in research and development of anti-cancer gene therapy, monoclonal antibodies, recombinant cytokines and clinical diagnostic products. The company has also founded a research center, Chengdu Center of Gene Technologies, focused on developing anti-cancer gene therapy products. The center has a 600 m2 pilot plant, and is able to produce 30,000-60,000 adenoviral particles per cell.

The anti-cancer gene therapy product, recombinant adenovirus mediated interleukin-2 to treat malignant cancer, has been approved by SFDA (possibly in February 2003). Currently, it has completed phase II clinical trails and obtained approval for its production, according to a report from Shenzhen Sci.&Tech. Expert Committee (http://www.szexpert.gov.cn/model/ dispnews.aspx?id=8&a0=113).

Guangzhou Doublle BioTech Co. Ltd.

Founded in May 2001 by Professor Wenlin Huang, Guangzhou Doublle Bioproducts Co., Ltd. (Guangzhou Doublle) is a biotech company engaged in the research, development, manufacturing and marketing of anti-cancer drugs. This company has its own capital and external funding. As it is aiming to expand commercializing and creating new product lines, it is calling for external investment.

Guangzhou Doublle BioTech has established a pilot production facility for the production of gene therapy drugs in Guangzhou Science Park. The facility houses 5 L and 43 L bioreactors. The company has plans to establish a GMP production line in 2010.

The company has developed a series of proprietary gene therapy products. Recombinant Human Endostatin Adenovirus Injection (E10A) is used for the treatment of solid tumors of head and neck, liver, pancreatic, lung, prostate, etc. It was approved by SFDA in December 2004 for a phase I clinical trial, and in February 2007 for a phase II trial. The development of E10A was partly supported by the National High Tech Program 863 Plan, the National SME Innovation Fund, and several provincial funds. Recombinant adenovirus carrying a human γ-interferon gene (E10B) has concluded its pre-clinical experiments and is waiting for the approval of SFDA for clinical trials. This product is expected to be used in the prevention and treatment of respiratory viral infections and of various malignant tumors. The recombinant oncolytic adenovirus injection (E10D) is being developed for treating ovarian cancer. Through injection, carrier cells infected with AdE3-IAI.3B, a replication-selective adenovirus for ovarian cancer, can enter ovarian tumor cells. Engulfed by tumor cells through endocytosis, the replication-selective AdE3-IAI.3B infects the host and undergoes proliferation, which leads to the dissolution, decomposition, and ultimately eradication of cancer cells.

Chengdu Kanghong Pharmaceuticals Group

Founded in 1994, Kanghong Pharmaceuticals Group is a medicine enterprise group headquartered in Chengdu, Sichuan, consisting of 7 member companies in Sichuan and Shanghai. The group is engaged in the preparation

and distribution of medicines in psychosis, neurosis, digestion, respiration, incretion and tumors. Currently, the group has 4 GMP production bases in the fields of natural medicines, chemical medicines, traditional Chinese medicines and chemical source medicines, and over 2,000 staff. The group covers an area of over 280,000 m^2 for the preparation of natural medicines, chemical medicines, and biological medicines. Its annual sales reach RMB 1 billion with the sales network ranging across all China's provinces, metropolitan areas, autonomous regions and southeastern Asia.

As a branch of the Group, Chengdu Kanghong Biological Science & Technology Co., Ltd. (Chengdu Kanghong), established in 2005, is a high-tech enterprise specialized in research and development of biotechnology pharmacy. The company has established three national-leading platforms, i.e., a large-scale virus production and purification platform, a large-scale protein and antibody production and purification platform, and a tumor vaccine production technical platform.

The company owns three newly developed medicines of independent intellectual property, including individualized tumor vaccine (KH901), an ophthalmologic genetic engineering agent (KH902) and tumor genetic engineering agent (KH903). Among those, KH901 is a gene therapy product, a recombinant adenovirus carrying the GM-CSF gene. The tumor cell-specific oncolytic virus kills tumor cells through specific replication, and produces an anti-tumor immune response by expressing GM-CSF to stimulate the immune system. It has concluded its phase I clinical trial, is approved by SFDA in September 2005, and is currently in phase II clinical trial.

OrienGen Biotechnology Ltd.

Oriengene Biotechnology Co. Ltd. (Oriengene) was founded in 2005 in Beijing Zhongguancun Biomedical Park by returned students. Oriengene is specialized in the development, manufacture and sale of Herpes simplex virus (HSV) vectors and gene therapy products. It is capable of up-to-pilot production and providing technical services for HSV users.

Oriengene is developing a gene therapy product, Recombinant Type I Herpes Simplex Virus Injection (OrienX010). This genetically modified oncolytic HSV-1 is carrying the GM-CSF gene to produce an anti-tumor immune response by expressing GM-CSF in tumor cells. Oriengene has obtained SFDA approval for phase I clinical trial in December 2009.

Telebio Lentiviral Vector Research Center

Telebio Lentiviral Vector Research Center was founded by Dr Guanghua Yang and Dr Wenwei Zhang in 2006 and is the first lentiviral vector product company in China. The company produces lentiviral vectors with a large-scale product system and purified Clinical Grade Lentiviral vectors. A viral titer of up to 10^{10} TU/ml can be obtained.

The company collaborates with EASCO (France) to organize a lentiviral vector training course in China. Televector™ biotechnology Co.,Ltd is a subsidiary company of Telebio, and provide several kinds of biotech support for Chinese Hospital and Institute. It offers services for gene overexpression and gene silencing as well as production of pseudotyped lentiviral vectors.

Hangzhou Yiyuan Biological Co. Ltd

Hangzhou Yiyuan Biological Co. Ltd. (Hangzhou Yiyuan) was established in 2007 in Hangzhou. The product Glucokinase mutation Gene therapy for Type II Diabetes is currently in preclinical evaluation.

Drug approvals in perspective

In the Western world the registrations of Gendicine and Oncorine were considered as exceptional. As a matter of fact, this holds also true for within China even though looking at raw numbers of drug approval in China one might believe otherwise. The year following the approval of Gendicine, some 10,011 new drug applications (NDAs) flooded into the SFDA and the vast majority were approved, according to Bill Liang, managing director of China Healthcare Consulting (Los Angelos, CA, USA). In comparison, approximately a 100 fold lower number of NDAs (i.e. 136) were approved by the US FDA during 2004 (Chenoweth 2005). This striking difference deserves a closer look, is China now really competing out the USA by having a much larger pharmaceutical or biotech output? This question has been investigated and described in an interesting News and Comment feature published in Drug Discovery Today (Chenoweth 2005). In both China and the USA, an NDA might be required for new uses of drugs already available. However, China is now in the process of registering, through NDAs, every drug now in use that was not previously registered, including the huge numbers of herbal and nature-based medicines that are part of the traditional Chinese medicine. This is one explanation for the different numbers. A second important difference that should be taken into account is the definition of a true new drug. In both countries, only a compound not previously approved for marketing as

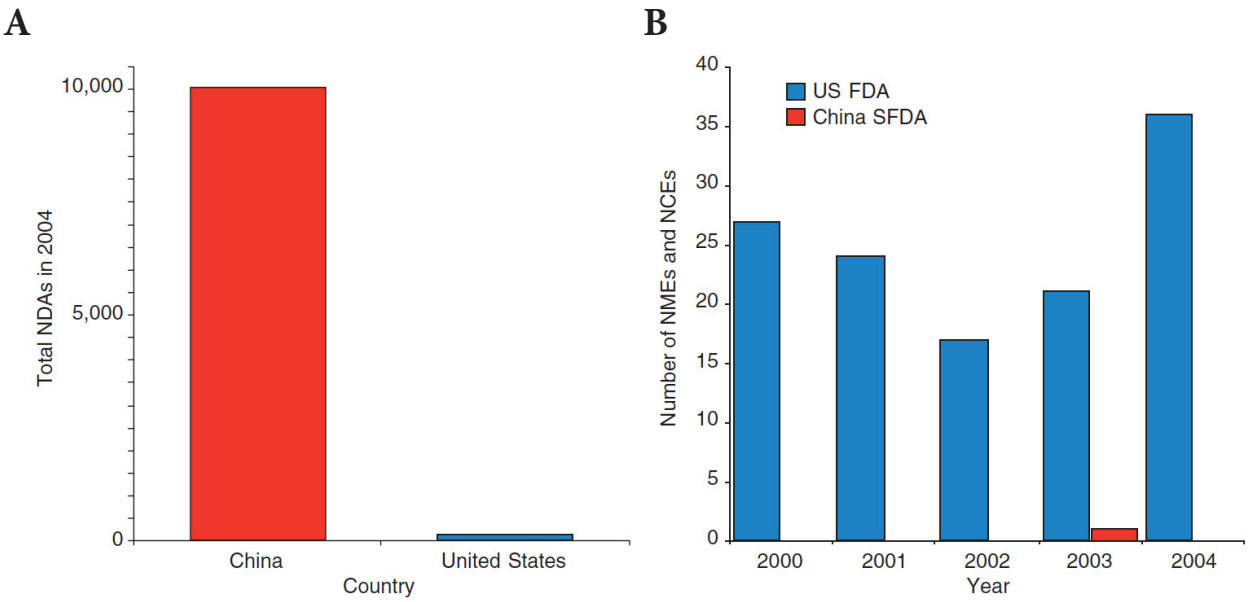


Figure 5.2. New drug applications in China and the USA. A Total NDAs approval in 2004, **B** NMEs and NCEs approved in period 2000-2004. Source: Chenoweth D (2005) Is more really less in China's new drug approvals? Drug Discov Today 1;10(17):1140-2.

a drug anywhere in the world is considered to be a true new drug. In China this is called a new chemical entity (NCE) and in the USA a new molecular entity (NME). According to Liang no NCE were submitted to the SFDA in 2004. Over 8500 applications included changes in dosage, route of administration or other new uses of drugs that were already within the Chinese pharmacopoeia, and some 1500 plus others were applications to register newly imported drugs. In contrast, however, 36 true new drugs, NMEs, were approved by the US FDA in 2004. Figure 5.2 taken from Drug Discovery Today (volume 10, number 17, 2005) gives a clear insight in the differences between China and the USA concerning new drug applications. Drugs like Gendicine and Oncorine are notable exceptions and if there were real new drugs the SFDA would make sure everyone hear about it according to Liang.

International collaborations

Academic initiatives

Exchange activities will often start most easily when Chinese researchers start to work at foreign academic institutes. It is generally known, but it also becomes clear from analyzing publications that these Chinese scientists are working in laboratories all over the USA (eg. University of Pittsburgh, University Florida, Boston University, Harvard University, see further Appendix). Many of the leading scientists in Chinese companies were formerly trained in the USA. In addition, the International Society of Cell and Gene Therapy for Cancer (ISCGT) organized meetings in China (Shenzhen, December 2005, Shijiazhuang, September 2008) to further enhance the opportunity for scientific exchange.

European connections include the Sino-German symposium on cancer research, held in Shanghai, May 2007 (Guo and Dietel, 2007). This symposium was supported by the Sino-Germany Science Center Beijing, the Deutsche Forschungsgemeinschaft and the National Science Foundation, China. Prof. Manfred Dietel, director of Institute fur Pathologie, Universitätsklinikum Charité der Humboldt-Universität Berlin, organized this meeting together with Dr. Yajun Guo. Yajun Guo, who worked for 7 years in the USA as an assistant professor, established in 1998 the Shanghai Zhangjing Biotechnology Center as an R & D research center for therapeutic antibodies. In 2005, he further developed the center into the present Shanghai Cell Engineering and Antibody Center, which consists of two academic institutions, SMMU and SJTU, and three

companies, CPGJ, LSGJ and ZJBC. His scientific interest focuses on experimental immunotherapy and immunogenetherapy.

In the UK, Prof. Nicholas Lemoine, director of the Institute of Cancer in London, actively contributed to the Sino-British Centre for Molecular Oncology, founded through a collaboration between Queen Mary University of London and Zhangzhou University in Henan Province in 2006. The Centre is developing viruses to treat cancer, and is funded through grants from the National Science Foundation and the government of Henan Province. The work is progressing through preclinical validations studies before clinical trials at Barts & The London School of Medicine & Dentistry and in Chinese centers will start. Furthermore, senior scientist dr. Yaohe Wang, who obtained his MD and PhD in China, joined the Lemoine group in July 2005.

In The Netherlands, a long exchange tradition has been in the vaccines area, Chinese scientists have been trained already many years ago at the National Institute for Public Health and the Environment (RIVM). Collaborations have been ongoing up to today with the Netherlands Vaccine Institute (NVI). Currently, the NVI is working together with Shanghai Institute of Biological Products and is responsible for the design and validation of a manufacturing site for bacterial vaccines in Shanghai (personal communication dr. Roeland van Dam, manager corporate development and licensing, NVI). Furthermore, The Netherlands is training many Chinese students, however, the exchanges at the more advanced level in the gene therapy field are currently still limited. Exchange activities between China and Europe related to the ethical aspects of clinical trials is described in the next chapter.

Industrial collaborations

On October 29, 2007 the Xconomy Boston announced on its website "Genzyme Forges Chinese Connection to Speed Gene Therapy to World Market". (www.xconomy.com/boston/2007/10/29/genzyme). This concerned a deal with Shanghai Sunway Biotech. Under the terms of the agreement, Genzyme's lead gene therapy candidate, Ad2/HIF-1a, would be jointly developed by Genzyme and Sunway in China (Genzyme would also continue to develop it on its own in the U.S.) The product promotes new blood vessel growth and is being tested in patients with circulation problems in their legs. The goal is to grow new vessels, thereby preventing the pain, disability, and need for amputation that may arise from this condition. Shanghai Sunway Biotech would produce for Genzyme and subsequently test it on patients in China. If the trials succeed, the two companies "envision jointly commercializing a therapy in China," according to a press release of Genzyme (not available any more).

6. SAFETY ISSUES

Historical overview

In 1998 the state food administration and the state drug administration merged into the Chinese State Food and Drug Administration (SFDA) to form the central agency to oversee the regulation, law enforcement and establishment of national standards. Since its establishment the SFDA has worked on raising the standards for its drug approval processes to comply with international norms and establishing itself as a credible regulatory body. However, this credibility is being hampered by food scandals. Moreover, in July 2007 the agency's former director, Zhang Xiaoyu, was executed after having pled guilty for the acceptance of bribes in exchange for approving drug production licenses. As a consequence, more than 170,000 production licenses issued by the SFDA are being reviewed, especially those approved between 1999 and 2002 (Jia, 2007).

Furthermore, questions have been raised about the fact that Dr. Peng of Shenzhen Sibiono GeneTech had participated in the drafting the "Points to Consider for Human Gene Therapy and Product Quality Control" (2003). However, scientists in other countries also work together with regulatory agencies, since they are experts in their specific field. Moreover, already in 1993 the Chinese Ministry of Health (MoH) published the document entitled "Points to Consider in Human Somatic Cell Therapy and Gene Therapy Clinical Research" as well as another guidance document in 1999 (Wilson, 2005, Zhang, 2008) [http://www.biopharm-mag.com/biopharm/article/articleDetail.jsp?id=95486 OR http://www.sfda.gov.cn/cmsweb/webportal].

In October 2004, relatives of a late-stage liver cancer patient sued Dr. Shanwen Zhang of Peking University's School of Oncology, because they said the off-label use of Gendicine had caused the patients' death. However, in January 2006 a medical accident appraisal committee in Beijing judged that the patient's death had nothing to do with the off-label use. Still, Dr. Zhang's hospital has stopped the off-label use of Gendicine (Jia, 2006).

It's been suggested that China was not affected by the death of Jesse Gelsinger in 1999 in the USA whereas in Western countries regulations were reevaluated. Actually, this tragic death resulted in the organization in the Beijing Xiangshan Summit Conference on gene therapy, organized by China's Ministry of Science and Technology. Both scientists and policy makers on human gene intervention took part in the Summit, in what turned out to be a very active and insightful debate on the ethical and regulatory issues. Some scientists supported a loose ethical review procedure for gene therapy clinical trials because a strict one would contribute to the loss of opportunity to catch up with the latest development in the field. Yet others thought that different protocols should be treated differently. If similar protocols have proven to be safe in other nations, Chinese Ethics Review Committees (ERCs) could shorten the tough review procedure. Notwithstanding the heated debate, there was a general consensus in the Summit that ethical review is a crucial step not only to protect the human subjects, but also for the good name of the nation (Zhang, 2008).

It is also important to realize that everywhere around the world scientists and doctors could be tempted to indulge in their wish to become the 'first in the world' instead of putting their patient's welfare first. This should be prevented and otherwise immediate actions should be taken. In 1980, Dr. Martin Cline performed DNA transfer into the bone marrow cells of two patients with hereditary blood disorders. He did so in direct opposition to National Institute of Health gene therapy guidelines and without the approval of the Institutional Review Board at the University of California Los Angeles (UCLA), where his research was conducted. The ethical concerns that were generated prompted a call for review by a number of organizations – including the National Council of Churches, Synagogue Council of America, and the United States Catholic Conference. Consequently, Dr. Cline was forced to resign his department chairmanship at UCLA and lost several research grants (Sun 1981).

The past has shown that incidents in the field of gene therapy can have a major impact on both the scientific and clinical developments as well as on the general opinion worldwide especially on safety aspects of gene therapy. Most important is the correct exchange of relevant information and prompt and proper actions taken by clinicians, scientists and regulatory authorities. Establishing collaborations and expanding exchange activities with China could prove to be most valuable. Currently, gene therapy activities in China do already have an impact on Dutch patients, even though this concerns limited numbers and is at the individual level. However, it is important to realize that future activities and/or possible incidents in China could play a major role in the progression of the gene therapy field in general and thereby affecting patient population at large. This could be irrespective of whether the gene therapy activities are comparable to activities pursued elsewhere in the world or not.

When comparing gene therapy performed in China to studies in Western countries, differences can be found,

but no real new strategies concerning gene vector technologies have been found in this study. In principle, the same risks for the environment can be anticipated as in Western studies. Below additional information is given on shedding data, cross-species medical interventions including genetically modified xenografts, medical tourism and ethical aspects of clinical studies performed in China that can be relevant to take into account in view of risk assessment.

Shedding data, biodistribution studies and toxicity tests

Only limited information could be retrieved from literature on shedding data, therefore relative more details are being given from individual studies rather than that a general overview of all clinical studies can be provided. Furthermore, results from biodistribution studies and toxicity tests in animal models have been added. Even though shedding was not a primary focus in animal experiments, the described studies can be informative for risk assessment.

Clinical studies

Adenoviral vectors

Adenovirus-p53

For the adenoviral vector containing the p53 gene that has been applied the most in Chinese hospitals (i.e. Gendicine), it has been difficult to get a better insight from clinical data due to lack of detailed information. Recently, this seems to be improving. Last year, there was more information reported on vector dissemination and biodistribution analyses. Gargle and urine samples of patients were collected just before vector injection and daily for 5 days after the first injection. Detection of viral DNA in these bodily secretions was rare. Only one sample of 30 serial gargle samples obtained from six patients and one sample of 23 serial urine samples obtained from five patients were positive. Furthermore, plasma samples were taken before, and 1, 2, and 24 hours after the first vector injection. In all eight patients tested, vector DNA was detected in plasma 1 and 2 hours after injection and negative at 24 hours after injection.

Adenovirus-endostatin

Biodistribution and vector shedding was studied more elaborately after intratumoral injection of a second generation, replication defective adenovirus containing human endostatin gene under the control of the cytomegalovirus immediate-early promoter (Ad-rhE or E10A) in patients with advanced solid tumors (Lin et al 2007, Li et al 2008). This was an open-label, non-randomized, dose escalation phase I clinical trial in which groups of three to six patients underwent all of the two-cycle planned doses, with one patient receiving only one injection.

Peripheral venous blood, injection-site swab, throat swab, urine and stool samples in relation to treatment were analyzed for the presence of vector DNA at the following time intervals: pre-treatment, immediately at the completion of virus injection, and 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hours after injection. Quantitative PCR using E10A-specific primer and probe combinations demonstrated that vector DNA was detectable in the injection-site swab in all patients immediately following intratumoral injection as well as 4 hours later. At 12 hours, one patient becomes negative at the site of injection. At 24 and 48 hours vector DNA detections are declining, at 72 hours only 2 patients still show positive samples and at 92 hours only one sample of one patient is still positive at the site of injection. Throat swabs are positive at 4 hours (11 out of 12 patients analyzed), 8 hours (9 out of 12), 12 hours (8 out of 12), 24 hours (7 out of 12), 48 hours (4 out of 12, all 4 receiving the highest dose), and at 72 hours 1 patient of the highest dose group is still positive at near threshold level (Li et al 2008).

Vector DNA was detected in urine (14 of 15 patients) and stool (7 of 15 patients) samples at low levels, but was non-detectable in any dose at 24 h post-intratumor injection of E10A as described in Li et al 2008. This is, however, in contrast to the paper by Lin et al 2007 where viral clearance was reported to be much slower in stool. Vector DNA was still detectable on day 7 after injection. 14 of 15 patients had plasma sampling of the detection of circulating E10A DNA. Vector DNA was detectable in the blood within 4 hours and peaked around 8 hours following intratumoral injection of E10A, declining to non-detectable levels after 24-48 hours for most patients. In one patient vector DNA was detected on day 7. No clear correlation between dose of E10A and the level and duration of vector DNA detectable in blood was observed, although there appears to be a general trend toward more circulating DNA and longer clearance time with higher doses (Li et al 2008).

In conclusion, distribution and shedding studies revealed that the vector was detected at the injection site, in throat swabs, in blood and stool, but only limited levels in the urine.

Conditionally replicating adenoviruses

Only limited data on biodistribution have been described for the H101, recombinant adenovirus in which the E1B-55kD gene has been totally deleted in addition to a deletion of the 78.3-83.8 MU gene segment in the E3 region (Lu, Zheng et al 2004). H101 5.0x10E11 virus particles were injected into the tumor for 5 consecutive days, and these injections were repeated every 3 weeks as one treatment cycle. Furthermore, all 50 patients were treated with routine systemic chemotherapy simultaneously. Overall, 46 patients were evaluated. Blood was taken before and one day after injections for PCR analysis. Sixteen patients were tested for plasma H101 viral genome before and 30 min after injection. Only six cases were positive after injections. All these patients were positive for blood adenovirus-specific neutralizing antibody on day 22. In total 14 patients were tested for the adenovirus-specific neutralizing antibody. Three of them were positive at baseline. Another 6 turned to be positive on day 22. No correlation was observed between baseline neutralizing antibody titers and induction of tumor response. Two patients who were positive at the baseline and two patients who were negative for adenovirus-specific neutralizing antibodies experienced tumor regression (Lu, Zheng et al 2004).

No biodistribution or shedding data have been found for H103 (containing the HSP70), the second virus of the Shanghai Sunway BioTech tested in the clinic (Li, Liu et al 2009).

Comparable to H101, circulation of KH901, a conditionally replicating oncolytic adenovirus containing granulocyte-macrophage colony-stimulating factor, have been reported. Moreover, for this vector the most elaborate shedding data have been described (Chang et al 2009). Blood samples were collected before and after KH901 injection (time 0, 45 min, 2 h, 12 h, 24 h, days 3, days 8 and days 15). The initial peak in circulating virus subsided to near-baseline levels in all patients tested within 12-24 h after treatment. Elimination of the virus appeared biphasic, with a sharp decline between 30 and 60 min after treatment and then a slower elimination over the next 12 h by first-order kinetics. A second peak was found in all patients tested (n=13) except three between 2 and 6 d after treatment. The viral load associated with this secondary peak varied between patients, and in at least four patients, it exceeded the load detected in circulation shortly after treatment. This secondary peak had a longer duration in most patients (median of 5 d) and, therefore, the total amount of systemic virus was substantially larger than that from the initial treatment in at least nine of 13 tested patients. For all patients tested, circulating KH901 levels returned to values indistinguishable from baseline by day 15 after treatment (Chang et al 2009). To investigate viral excretion, KH901 was measured in samples taken from the pharynx, urine and feces before and after KH901 injection. By day 2 posttreatment, urine from four of 23 treated patients was positive for viral shedding as determined by plaque formation at > 50 plaque-forming units/mL. Urine from only two patients continued to induce plaque formation at day 8, and days 15 and 22. Urine samples became positive for KH901 1 d following KH 901 injection and remained positive in 95% of urine samples 15 d following treatment. Most of feces and pharyngeal samples were negative for KH901 from day to day15, while one feces and one pharyngeal specimen were positive (Chang et al 2009).

Animal studies

Adenoviral vectors

The lab of Huang investigated the dynamic distribution and expression in vivo of recombinant

human endostatin gene delivered by an adenoviral vector (Ad-rhE; E10A) using fluorescent real-time quantitative PCR and enzyme-linked immunosorbent assay, respectively. BALB/c nude mice (3 male, 3 female) were single administered intratumorally with 2.0x10E9 pfu Ad-rhE. The tissue samples of heart, liver, spleen, lung, kidney, gonad, brain, and tumor were harvested on days 1, 3, 5, 7, and 11 post-injection of Ad-rhE. The obtained results showed that Ad-rhE DNA in tumors is highest at day 1 (over 400,000 copies/mg tissue), had decreased sharply by day 3 and remained at the same level for days 5 and 7 (10,000–20,000 copies/mg tissue), and lingered in lower concentrations at day 11. Ad-rhE DNA was also detected in blood, liver, kidneys, and spleen with the highest levels at day 3 and 5. Furthermore, lower levels of Ad-rhE DNA were detected in heart and lung as well as gonads. The concentrations of endostatin showed the same trends, however, the differences between tumor samples and other tissue samples were less profound. Highest concentrations were observed at day 5 (30 ng/mg tumor, 22-27 ng/mg heart, 25 ng/µl blood, and 5-12 ng/mg for gonads, kidney, spleen, lung, brain). However, no specific comments were given on shedding aspects (He et al 2005).

Moreover, in contrast to the details described for the clinical study with E10A, in the long-term toxicity studies in beagle dogs (12 male and 12 female) only very limited information is given on biodistribution and no data were

given for shedding of the viral vector. Intramuscular injections of E10A at high and low doses equivalent to 30 and 7.5 times the human curative dose, respectively, were given once daily, 6 days/week, for 3 months, followed by a 1 month recovery period. The studies were focused on toxic effects, eg. urinalysis, hemogram blood biochemistry, electrocardiogram, macroscopic and microscopic studies of organs and tissues were done before treatment, at month 3 of treatment and 1 month post treatment. Intramuscular injection transferred genes locally and fewer adenoviruses entered blood circulation, reducing toxicity to vital organs, particularly hepatic toxicity. No significant abnormal toxic effects were reported. Anti-adenoviral antibodies were generated in a dose- and time-independent manner after E10A injection (Huang et al 2007).

Two years later, the same laboratory, i.e. the State Key Laboratory of Oncology of the Sun Yat-Sen University, gives some more information on biodistribution of an adenovirus expressing the human interferon gamma gene (Ad-IFN-γ). After intratumoral administration of 1x10E10 VP/tumor Ad-IFN-γ into CNE-2 xenografts, a tumor model in BALB/c nude mice, samples of blood, tumor, heart , liver, spleen, lung, kidney and brain were harvested on days 1, 2, 3, 5, 7, 14 and 21. Ad-IFN-γ DNA was mainly detected in tumors where the Ad-IFN-γ was injected ($p<0.05$, compared to blood or parenchymal organs), then in livers ($p<0.05$, compared to blood or other parenchymal organs). Concentrations of Ad-IFN-γ DNA were very low in other organs and blood. Ad-IFN-γ DNA lasted for at least 7 days in the tumors. Ad-IFN-γ DNA in livers decreased in a time-dependent manner and became undetectable at day 14 after intratumoral administration.

Conditionally replicating adenoviruses

Several different animal models were used to test the toxicity of a triple-regulated gene oncolytic adenovirus (SG600-p53). The human adenovirus type 5 was modified by a partially deleted E1a under the control of the promoter of human telomerase reverse transcriptase (hTERT), and the E1b promoter was replaced by a cis-element of five copies of hypoxia regulatory element (HRE). The p53 gene under control of the CMV promoter was inserted between E1a and E1b genes. Safety pharmacology tests were done in mice and cats, the latter especially for testing the effects on cardiovascular and respiratory systems. Acute toxicity tests were done in mice. Maximum tolerated dose (2.5x10E13 VP/kg) induced cachexia, decreased activity and eye closure in 9/20 mice which could be self-resolved within 30 minutes. Systemic anaphylaxis testing in guinea pigs did not show any adverse effect. Rats were used for repeated dose toxicity tests, in which the partially deleted E1a and E1b genes are regulated under the human telomerase reverse transcriptase promoter and the hypoxia response element.

Conclusions shedding data

Up to now only limited shedding data from Chinese studies could be retrieved. From these data is becomes clear that shedding does occur and it has been suggested that this is dosage dependent. Especially, positive samples of throat swaps, blood and stool during the first days after virus injection indicate the importance of having the proper trained personnel in order to prevent transmission of viruses from the patient into the environment, especially to other persons. Since several clinical gene therapy studies use a protocol with injections of the viral vector into the patient at multiple time points, there are several occasions during the therapy of one patient that an incident of viral spread into the environment could occur. The experience and knowledge of the caretakers of patients has not been further investigated.

The risk of shedding could potentially increase with the use of selective replicating adenoviruses since the total amount of viruses could be augmented. The occurrence of shedding has not been studied in more detail for these types of viruses, an observation that has also been made when analyzing reporting of shedding in Western studies (Schenk et al, 2006).

There appears to be a trend over time that there is increasing attention to shedding aspects. This positive development could be noticed over time with the initiation of new clinical trials (eg. KH901, a conditionally replicating oncolytic adenovirus containing granulocyte-macrophage colony-stimulating factor) as well as within already existing research groups (eg. State Key Laboratory of Oncology of the Sun Yat-Sen University).

Finally, it is important to realize that shedding data documented in clinical studies in China do have the potential to be an informative resource to Western scientists due to the fact that many more patients have been treated. Also the selection criteria appear to be different, with more patients admitted in an earlier disease stage as well as having more combination therapies that could influence the outcomes. The overall conclusion could be that China harbors a potential pool of information that seems to be lost for the first clinical trials, but that there are indications that this is changing for the better. Obviously, this would be beneficial also for clinical gene therapy performed in the rest of the world as well.

Germline transmission

Transmission of DNA to the germ line in clinical gene therapy studies is an adverse side effect that should be prevented at all costs. Up til now, germline transmission has not been reported in Chinese gene therapy clinical studies. However, the majority of the treated patients concerned terminally ill patients and information on shedding data in general is also limited. On the other hand, studies have been performed with the deliberate aim to obtain germline transmission. An article is written by Niu and Liang of the Laboratory Animal Center, Peking University People's Hospital, on "Progress in gene transfer by germ cells in mammals" (2008). A recent example is human Factor VIII transgenic mice generated by DNA transfection of sperm isolated from caudea epididymides of male C57BL/6 mice (Yin et al 2009). This type of research has also been described for exploring options for the treatment of male infertility in other countries (Coward et al 2007, Kojima et al. 2008).

Up to now there is an international consensus on a halt to germline gene transfer and this position is also being taken by Chinese scientists. Another area of research that must still be debated before clinical practice can become a reality is in utero gene transfer. The group of Dr. Xue in Shanghai, who also performed the first clinical trial for hemophilia, published an article on "Efficient delivery of human clotting factor IX after injection of lentiviral vectors in utero" (Acta Pharmacol Sin 2004). In this mouse study, the authors also looked at germ line transmission which could not be detected at the DNA and RNA level. hFIX cDNA was detected in liver, spleen and heart. For now there is no clinical study reported involving in utero gene transfer being performed in China, based on information retrieved from the SFDA database, nor is one expected to be conducted in the near future.

Genetically modified xenografts

In 2006 Chongqing Zongshenjunhui Biotech (Chengdu) launched Tiefu™ as an innovative biological wound dressing. Their products are made by genetic modification technology using fresh pigskin as the primary material. The pigskins are harvested from pigs with identical genetic and biological characteristics. Subsequently, an adenoviral vector containing the CTLA4Ig gene is used for transduction in order to inhibit the T cell response from the host against the graft material. The obtained modified cells are used primarily for effective wound covering for a prolonged period to prevent infection as well as water loss, and to promote wound closure. National Key Scientific Project, Chongqing Municipal Key Scientific Project and Innovation Fund for Small Technology-based Firms have all supported the R&D of this product. In addition, the People's Liberation Army awarded Tiefu™ with the second-degree award for scientific technology development. (http://www.zongshenbiotech.com/english/cp/cp.asp). This product is considered a medical device and is not defined as gene therapy. Still, in view of risk assessment it will be of interest to monitor the alternative use of genetically modified viral vectors and the broader availability of genetically modified materials. In addition, the application of cross-species medical interventions should be done with caution.

Pig material has already been in use for some years by cardiac surgeons. Pig valves can function as spare parts for the human heart, however, these operations are not regarded as xenotransplants as the valve is regarded as inert rather than living tissue. In contrast, the transplantation of organs, such as heart and kidney, or pancreatic islet cell transplantation to treat type I diabetes could increase the risk of pathologies crossing the species barrier. It will be most relevant to have sufficient knowledge about endogenous and exogenous viruses that could be present in the donor and host, especially when using viral vectors for the genetic modification of transplanted material.

Prof. Wei Wang has reported on intrahepatic transplantation of porcine islets to four patients with type I diabetes (in Chinese: Journal of Chinese Physician 2003, 5, 10). Five years of follow up did not show evidence of porcine origin viral infection in patients. Obviously, monitoring for viral spread across species remains important. Recently, Xenome, a Framework 6 EU-project has been devoted to xenotransplantation and scientific information as well as legal and ethical information can be retrieved from the project website (http://www.xenome.eu/new_start.aspx). In the appendix some of the main issues of the project, especially related to the risk of unwanted virus spread, have been listed.

Medical tourism

The easy access to information on new treatments available on the internet will attract the attention of people that have exhausted the conventional treatment options and are looking for a ray of hope. In 2005, Gendicine was advertised by a Canadian pharmacy website telling consumers: "Doctors are now extending the treatment, named Gendicine, to patients with lung and stomach cancer. Injected directly into the tumor, Gendicine works by, in effect, programming cancer cells to commit suicide. About 400 patients so far have been treated with the drug in eight-week courses which cost the equivalent of 1,800

pounds." (Krimsky, 2005). Currently, this information is no longer available. Furthermore, in 2007 a Danish television program followed Danish patients that were undergoing Gendicine treatment. In different publications as well as in personal contacts the number patients traveling from abroad (USA, Germany, Denmark, Thailand, the Philippines, Greece, Canada, the UK, Singapore, Russia, Rumania and Turkey) to China is always given at 300 to 600.

Exact numbers of Dutch patients are not known, but it is certain that several patients have traveled from The Netherlands to China for Gendicine treatment. On the website of Cancer Therapy China (www.cancertherapychina.com), short reports can be found of five Dutch patients who traveled from The Netherlands to China to undergo cancer treatment. More details of these patients are given at the end of Chapter 7: Relevant Websites. These reports are mostly dated 2005- 2007, the period when Gendicine received the most publicity.

The Netherlands umbrella organisation for cancer patients (Nederlandse Federatie van Kankerpatiëntenorganisaties) has not been approached by cancer patients during the past years to inquire about the possibility for gene therapy or stem cell therapy in China. Currently, the NFK would advice patients not to travel to China, South Korea etc for these kinds of therapies. Still, occasionally enquiries to doctors and scientists are made by patients in The Netherlands on how to get into contact with Chinese doctors for Gendicine treatment.

Information on foreign patients treated with Oncorine is hard to find. However, this could change, since PolarisRx Corporation (Kobe, Japan) started to advertise for PRX-100 E1b 55kd deleted adenovirus therapy (Oncorine) in February 2010**.** Obviously, the more publicity given to a new treatment being offered, the more patients this will attract (see also chapter 7 – relevant websites). Based on what is known so far, it would not be advisable for patients to travel to China for gene therapy cancer treatment, since no convincing data are present that show efficacy. In chapter 1, the introduction, the numbers of patients traveling to China for stem cell treatment and gene therapy were compared. Even though the numbers for stem cell treatments are currently 4-fold higher, access to gene therapy treatments for foreign patients could easily be expanded since the drugs can be exported, whereas undergoing stem cell treatment is more difficult and less prone to be 'shipped' to other countries. The lower patient numbers are therefore not representative of possible consequences of further developments in the gene therapy field as compared to the stem cell field. Moreover, stem cell treatment will only have consequences for the treated individual, whereas gene therapy treatment could potentially also harbor risks to the environment of the treated individual. Even though this might be a small risk, it is important to be aware of consequences. This is especially true when patients do not communicate their alternative treatments to their regular physician(s) and treatments are being combined without proper knowledge.

Ethical aspects of clinical trials

The European Commission has acknowledged the challenges facing the ethical governance of research in the life sciences and biomedicine in China as well as in the EU and therefore funded the BIONET consortium, a Framework 6 project. This project consisted of a total of 21 EU and Chinese partners that focused on three key areas: reproductive and regenerative medicine, clinical trials and biobanks and the genomics of disease susceptibility and treatability. The results of this work are documented on the project website (http://www.bionet-china.org/).

A concluding report was published on September 4, 2009 stating that vulnerable patients who travel abroad for unproven and potentially unsafe stem cell treatments need to be better protected. The report calls for countries to develop more effective regulation of experimental stem cell procedures by insisting on rigorous clinical studies and ethical reviews before they can be offered as treatments. Proposals have been published by the Expert Group of the BIONET consortium, a group of 10 Chinese and European experts, from the fields of medicine, ethics, law, political science and social science. This Expert Group is an independent part of the BIONET project, a Sino-European collaboration based at the London School of Economics and Political Science (LSE). "The growing global stem cell tourism economy has been fuelled by claims of treatments for hitherto untreatable conditions, the formation of patient networks, falling travel costs and the establishment of high quality medical facilities combined with undeveloped or non-existent national regulations. While most stem cell therapies have not undergone clinical trials, clinics throughout the world, including Europe, Asia and the Americas, are offering patients - who are often extremely vulnerable and have exhausted all other options - expensive and unproven treatments." Qiu Renzong, Professor of Bioethics at the Chinese Academy of Social Sciences and the co-chair of the BIONET Expert Group, said: "Stem cell research is tremendously exciting and may lead to potential treatments. However its development must be governed in an ethical and respon-

sible way if it is to fulfil its potential and not experience a backlash from public opinion. Many countries, including China and those in the EU, are now starting to regulate these therapies. However, if patients are to be properly protected, regulation needs to be enforceable and effective." The BIONET Expert Group's report makes a number of other recommendations on Sino-European research collaborations, including greater clarity and more precise regulation of clinical trials, international agreements about the ethics and transparency of biobanking - the storing of human blood and tissue for research purposes - and the establishment of a permanent China-Europe partnership on research ethics.

Even though the project is not focused on gene therapy, the conclusions could be extrapolated to new emerging therapies in the biotechnology field in general. Like stem cells, gene therapy also holds the promise of treating a disease in a novel and fundamental way and attracts patients that have no other options left. Despite critical comments on neglect of ethical issues, it is noteworthy to mention that a Chinese Ph.D. thesis was especially devoted to the Ethics of Gene Therapy. An English version by this author on this topic is available (Zhang, 2008).

Overall conclusions risk assessment

- Shedding does occur
- Especially in view of spread of viruses into the environment is most critical to have well trained doctors as well as nurses in these studies
- It is not further investigated what the experience is of the caretakers of patients
- New type of risks could be anticipated, even though this is not exclusively for China, still opportunities might be higher. Therefore important to monitor cross-species treatments in order to anticipate on potential transmission of infectious agents crossing species borders.
- Medical tourism does occur in the gene therapy field, including Dutch patients traveling to China for gene therapy treatment
- Germline transmission is not studied extensively for these report, but there appears to be no indication to have special concerns related to this issue

7. OVERALL OBSERVATIONS AND CONCLUSIONS

Introduction

In 1991 the first clinical experiments were started with gene therapy and the expectations in the scientific community and the public at large were high. Now in 2010, almost 20 years later, no gene therapy product has received a marketing authorisation in the EU and US. In the EU the EMA is as yet not convinced about a positive risk / benefit ratio of these therapies and no positive opinion has been issued up to this moment. This is in contrast with the development in China where in 2003 a first product (Gendicine®) for the treatment of cancer obtained a positive opinion and marketing authorisation, followed in 2005 by a second product (Oncorine®). This development has not gone unnoticed by cancer patients, some times desperately searching for a cure where ever in the world. Among the thousands of patients treated in China with these gene therapy products of viral origin are many 'medical tourists' from other countries, among them also from the EU and The Netherlands. So The Netherlands may be faced with patients returning from China after treatment with viral gene therapy. Potentially posing an environmental and health risk, an d thus of concern of health and environmental authorities.

This project was initiated to find answers to the following questions related to developments in China:

1. What are these approved gene therapy products, and do they pose a (public) health and environmental risk for The Netherlands?
2. What are the current developments in the field of gene therapy in China?
   - What type of gene transfer technologies are being investigated and may be in clinical development later?
   - What other diseases than cancer are under (clinical) investigation?
3. How can we learn about these developments?
   - What sources are accessible (in English) and what sources are only accessible with proper knowledge of the Chinese language?
   - Could we benefit from the experience collected in clinical trials., e.g. efficacy, safety and shedding data?
4. What could be the implications for patients, public health and environment?

After the start of the project it soon became clear that input by Chinese scientists was most valuable. The merits of Chinese literature databases were put into perspective by indicating the relative value of the publications listed. Chinese scientists prefer to use English literature databases, especially PubMed, to find the most relevant literature, also when evaluating results from their colleagues in China. Still, these efforts were pursued to gain further insight in Chinese scientific literature and possibly additional confirmation of these annotations. At the same time, it was felt necessary to explore additional resources both from Chinese origin (searching Chinese websites, attending meetings in China) as well as Western origin (searching Western literature databases, internet) to achieve a complete overview of gene therapy developments in China.

Developments in gene therapy field

Gene therapy research in China is widespread, extensive and rapidly growing

From analyses of Chinese literature databases (CNKI, Wanfang and CQVIP) and Western literature databases (ISI Web of Science, PubMed) the following observations and conclusions could be made:

- Chinese literature databases show an approximately 2-fold increase in gene therapy publications from 2000 to 2006, while from 2007 onwards the growth in numbers in Chinese language is slowing down.
- Western literature databases show an approximately 3-fold increase in publications of Chinese authors on gene therapy from 2000 to 2004, whereas from 2005 the number increase at a more rapid rate through 2008 to stabilize in 2009.
- The results taken together show a) an overall increase of publications by scientists from China and b) a shift from Chinese to Western literature databases. Taken together this means that the output from gene therapy research is increasing and is becoming more transparent for those not familiar with the Chinese language.

Research activities are as broad as in the rest of the world

The most complete and informative Chinese literature database for gene therapy, i.e. the CNKI database, provided information on the type of publications, on the relative share of various disease areas studied, as well as on vector technologies:

- The majority of publications on gene therapy that we do not have access to in Western databases turn out to be Ph.D. theses, indicating that a large contingent of Chinese students are being trained in the gene therapy field.

- Analyses of the CNKI database over three time periods (1996-2000, 2001-2005 and 2006-2010) displayed the prominent position of cancer (starting from 40% in the first period up to 50% in the last period), followed by liver related disorders (from 6% up to 11%), neurological disorders (from 7% up to 11%), cardiovascular diseases (from 3% up to 6%), and diabetes (1-2%).
- Similar analyses of trends over time were done for vector technologies. Non-viral approaches (especially RNA technologies and lipofection) are described in 19% up to 43% of the studies from 1996-2000 with the most recent five years. Viral vector mediated gene transfer studies show a small decrease from 31% to 29%. The adenoviral vectors are still popular and show an increase from 12% to 17%. Conventional retroviral vectors are studied significantly less (14% to 4%), the decline most likely favours the use of lentiviral vectors (0.2%-2.8%). Herpes simplex virus (2.7-1.6%), poxvirus (0.1%), Sendai virus (0.1%), vaccinia virus (0.3-0.1%) are hardly studied and declining further. Traditionally, Chinese scientists publish many reviews, in the vector technology representing 14% to 19% of the total number of publications.
- In terms of topics, research in China is as broad as in the rest of the world. As might have been expected a prominent focus is apparent to disease areas that have a relative high prevalence in China, e.g., liver related disorders (hepatitis, liver cirrhosis).

Clinical studies are increasing in recent years

The search of the website of the Chinese State Food and Drug Administration and other Chinese websites, combined with literature searches from Chinese and English databases, in addition to personal contacts provided the following information on clinical studies and industrial activities:

- The total number of clinical studies approved in China that could be retrieved was 44. The first trial was approved in 1991, which parallels world-wide activities. However, the next studies were started at a relative slower rate when compared to the rest of the world (up to 2000 6 studies in total) and showed an increase from 2000 onward (2-6 studies per year).
- The majority of clinical studies concern cancer patients. Others include cardiovascular and ischemic diseases, hepatitis B, and hemophilia B.
- At least ten companies in China are directed at gene therapy and recently two more have been established. Five companies are directed at genetic vaccines, another company, Chongqing Zongsheng Biotech (Chengdu), has launched Tiefu™ (Ad-CTLA4Ig expressed in pig skin cells) in 2006 for the treatment of skin wounds.
- It is concluded that the clinical activities in China are increasing relative to the rest of the world. More companies are active than were known so far and are covering a broad spectrum of activities.

Clinical practice

Thousands of patients are being treated in China with gene therapy products.

- Anecdotal information has been found on five Dutch patients that received Gendicine® treatment in China. It was also suggested by a Chinese doctor to a Dutch patient to be receive Gendicine® treatment in The Netherlands. Several websites have been found that have the clear aim of attracting foreign patients for gene therapy treatment in China.
- The total number of patients undergoing gene therapy treatment in China is much higher than in the rest of the world, due to the registration of Gendicine® and Oncorine®. These products have also been in use for off-label applications.

Safety concerns

We can assume that the overall potential risks of gene therapy in China are similar to those of gene therapy world-wide, as similar types of vectors are used for similar types of diseases, although we lack specific information. Nevertheless, there are also safety concerns which are more specific for China. In addition, some issues are listed that are relatively new, but not specific for China.

New issues in some aspects unique for China up to now

- The advent of internet offers every patient a world wide view on possible therapies, including gene therapy treatment offered by Chinese hospitals.
- The resulting medical tourism for gene therapy treatment in China is unprecedented. Even though the numbers of patients that have travelled to China appear to be relatively small, gene therapy treatment could not only have consequences for the treated individual, but also potentially can also pose a risk to the environment of the treated individual. At this moment, we estimate currently this risk small. Especially when patients do no communicate the treatment with a gene therapy product to their regular physician(s), patients may receive concomittant standard chemotherapy. Many of the standard cancer chemotherapy schemes suppress the immune system with a poorly

known outcome after a previous treatment with a viral gene therapy product.
- The introduction of the innovative biological wound dressing Tiefu™ had almost gone unnoticed and demonstrates that broader application of viral vector technology has become a reality. This product is considered a medical device and is not defined as gene therapy. The used transgene, CTLA4Ig, has an immune suppressive effect. Obviously, it would be undesirable to introduce genes into the environment that could exert an inhibitory effect on the immune system of the general population.
- China potentially could have the largest database with clinical data from gene therapy patients. Recording and publication of these data, including shedding data, should be encouraged to enable future risk assessments.

Other noteworthy topics related to risk assessment
- It has been suggested that China was not affected by the death of Jesse Gelsinger in 1999 in the USA whereas in Western countries regulations were reevaluated. Actually, this tragic death resulted in the organization in the Beijing Xiangshan Summit Conference on gene therapy, organized by China's Ministry of Science and Technology. There was a general consensus in the Summit that ethical review is a crucial step not only to protect the human subjects, but also to protect the reputation of the nation.
- The application of Tiefu™ does not only harbor some risk of undesired viral transmission of the applied adenoviral vector, but the use of pig skin entails a cross-species intervention with other specific risks and should be done with caution to prevent transmission of animal viruses to man. Sufficient knowledge is required on endogenous and exogenous viruses that could be present in the donor host, especially when using viral vectors for the genetic modification of transplanted material. Xenotransplantation (e.g., pancreatic islet cell transplantation to treat type I diabetes) is also investigated in other countries. The issue of transmission of viruses crossing the species barrier is therefore not unique for China, but still is an important issue in view of the registration of a gene modified xenotransplantation product.

In conclusion

The information on gene therapy in China described in this report is a 2010 snapshot. We have shown that gene therapy research in China rapidly expands, so it can be expected that the picture may be very different in 5 years time. Therefore, continued monitoring of its further development is mandatory. We recommend an international collaboration for this on a EU level.

8. RELEVANT WEBSITES

State Food and Drug Authorization
http://eng.sfda.gov.cn
Main Responsibilities
1. To formulate policies and programs on the administration of drugs, medical devices, health food and cosmetics, as well as food safety at consumption stage (restaurant, cafeteria, etc.) and supervise their implementation; to bear a part in drafting relevant laws, regulations and normative documents;

5. To take charge of administrative and technical supervision of drugs and medical devices, take charge of formulating good practices for drugs, medical devices in aspects of research, production, distribution and use, and supervise their implementation;

6. To take charge of registration and supervision of drugs and medical devices; draw up relevant national standards of drugs and medical devices, and supervise their implementation; carry out the Adverse Drug Reaction (ADR) monitoring and adverse event monitoring of medical devices; be responsible for drug and medical device re-evaluation and elimination; bear a part in formulating national essential medicine list and adopting the national essential medicine system, and organize the implementation of classification system for prescription drugs and non-prescription drugs;

>>> Short explanation on this website is given at the end of this list of relevant websites.

Ministry of Science and Technology of the People's Republic of China
http://www.most.gov.cn/eng/index.htm
China Science and Technology Statistics Data Book People's Republic of China (2007)
http://www.most.gov.cn/eng/statistics/2007/index.htm

CHINESE LITERATURE DATABASES:

China National Knowledge Infrastructure (CNKI)
http://www.global.cnki.net/grid20/index.htm
China National Knowledge Infrastructure (CNKI) is a key national project of China. Its purpose is knowledge sharing throughout China and the world. From its beginning in June, 1996, the global reach of CNKI full-text databases has grown substantially. CNKI now serves more than four hundred universities, public libraries, research institutions, enterprises, and hospitals in more than twenty countries.

Wanfang database
http://www.wanfangdata.com/index.asp
As an affiliate of Chinese Ministry of Science & Technology, Wanfang Data has been the leading information provider in China since 1950s. With a wide range of database resources and value-added services, Wanfang Data has become a gateway to understand Chinese culture, medicine, business, science, etc.

Chongqing VIP Information Co., Ltd – only in Chinese
http://www.cqvip.com/
Chongqing VIP Information Co., Ltd is a subsidiary of S&T Department-Southwest Information Center. Its online databases have collected 400 Chinese newspapers, more than 8,000 Chinese journals, 5,000 foreign journals. It is a Chinese Scientific Journals Fulltext Database, which is the biggest national comprehensive documentary database, covering 8,300,000 documents that were published in more than 8,000 periodicals since 1989.

COMPANIES IN CHINA RELATED TO GENE THERAPY:

Shenzhen SiBiono GeneTech Co., Ltd.
http://www.sibiono.com/en/introduce.html

Shanghai Sunway Biotech Co., Ltd.
http://www.sunwaybio.com.cn/index.html

Beijing Vector Gene Technology Company Limited
http://www.agtc.com.cn/agtc_en/en/index.html

Shenzhen Tian Da Kang Gene Engineering Co. Ltd.
http://www.ship.gov.cn/index.asp?bianhao=9598

Royal (Wuxi) Bio-Pharmaceutical Co., Ltd.
http://www.chinaroyal.cn/, or http://www.rbpc.com.cn

Chengdu Hoist Group Co., Ltd.
http://www.licartin.com/newEbiz1/EbizPortalFG/portal/html/index.html

Guangzhou Doublle BioTech Co.,Ltd
www.gzdoublle.cn

Chengdu Kanghong Pharmaceuticals Group
http://www.cn-kanghong.com/

OrienGen Biotechnology Ltd.
http://oriengenebio.com.cn/

Telebio Lentiviral Vector Research Center
http://www.televector.com/english/index.asp

Chongqing Zongshen Biotech
http://www.zongshenbiotech.com/
Companies in China related to genetic vaccines:

Changchun Baike Pharmaceutical Inc Co., Ltd.
http://www.zongshenbiotech.com/

Beijing Tiantan Biological Products Co., Ltd.
http://www.tiantanbio.com/

Guangzhou Baidi Biotechnology Co., Ltd.
http://www.gzbiotech.com/

Shenzhen Tsinghua Yuanxing Pharmaceutical co., Ltd.
http://www.thyx.com/

Shanghai Fudan Yueda Biotech co., Ltd.
http://www.fudanyueda.com

WEBSITES ATTRACTING PATIENTS FOR GENE THERAPY TREATMENT IN CHINA:

Cancer Therapy China
http://www.cancertherapychina.com/
Cancer Therapy China is a website sponsored by China Cancer Centers Limited (CCCL).
"Our goal is to be the definitive source for information, consultations, and the provision of a range of cutting edge cancer treatments currently available or pending authorization in China. We also strive to provide professional guidance to victims of cancer in understanding, accessing, and receiving these treatments, some of which are unavailable or experimental in the Western world."

This website also contains information on Dutch patients treated with Gendicine, see at the end of this chapter.

GreatWall International Cancer Center (Beijing, China)
http://genetherapyhospitals.com/genetherapy.html
This website provides information on Gendicine treatment in China, including patient stories.

Phoenix Cancer Center (Beijing, China)
Gene therapy & biological medicine for cancer
http://www.phoenixcancercentre.com/treatments/gene-therapy
This website provides information on Gendicine and Oncorine treatment in China, including patient stories.

PolarisRx Corporation (Kobe, Japan)
http://www.polarisrx.co.jp/recruit.html
In February 2010, PolarisRx Corporation started to advertise for **PRX-100 E1b 55kd deleted adenovirus therapy (Oncorine).**

INFORMATION ON FUNDING:

973 National Basic Research Program of China
Applied Basic Research on Gene Therapy http://www.973.gov.cn/English/ReadItem.aspx?itemid=485

China Scholarship Council
http://en.csc.edu.cn/
The China Scholarship Council (CSC) is a non-profit institution with legal person status affiliated with the Ministry of Education. The objective of the CSC is to provide, in accordance with the law, statutes and relevant principles and policies of China, financial assistance to the Chinese citizens wishing to study abroad and to the foreign citizens wishing to study in China in order to develop the educational, scientific and technological, and cultural exchanges and economic and trade cooperation between China and other countries, to strengthen the friendship and understanding between Chinese people and the people of all other countries, and to promote world peace and the socialist modernization drive in China. China Scholarship Council is financed mainly by the states special appropriations for scholarship programmes. At the same time the CSC accepts donations from the personages, enterprises, social organizations and other organizations at home and abroad.

OTHER INFORMATIVE WEBSITES:

Gene therapy and China on Gene Therapy Net
http://www.genetherapynet.com/asia/china.html

Cultural issues in Bioethics
http://www.ruhr-uni-bochum.de/kbe/
Kulturübergreifende Bioethik > Publikationen > China Publications on Bioethics & China

BIONET

http://www.bionet-china.org/
BIONET is a Consortium of 21 EU and Chinese partners that has examined the challenges facing the ethical governance of research in the life sciences and biomedicine in China and the EU. It was funded under the European Commission Sixth Framework programme with support from the United Kingdom's Medical Research Council. As a sustainable network within and between China and Europe, BIONET focused on three key areas: reproductive and regenerative medicine, clinical trials and biobanks and the genomics of disease susceptibility and treatability. The BIONET project has now drawn to a close. Over three years (2006-2009), BIONET examined challenges of ethical governance related to Sino-European collaboration in advanced life sciences research. The results of this work are documented on this website. Publications by BIONET members (English): http://www.bionet-china.org/pub_members2.htm

XENOME PROJECT

http://www.xenome.eu/
Xenome is an integrated project in the European Sixth Framework Programme (Life Sciences, Genomics and Biotechnology for Health).
Mission:

- Establish the EU at the forefront of global xenotransplantation research, a field with huge potential for the treatment of end-stage organ failure. At the end of the programme, the project aims to deliver a European "product" for xenotransplantation.
- Generate in vivo efficacy data and provide experimental evidence that "engineered" pigs, combined with new immunosuppressive reagents, allow long-term survival of porcine organs transplanted in non human primates.
- Assess new immunosuppressive regimens and agents for efficient inhibition of immunological rejection. Gain an understanding of the physiology of long-term transplanted xenografts in primate recipients.
- Establish the necessary safety framework to allow progression of xenotransplantation to the clinic
- Provide a strong ethical, social, educational through direct engagement of the public) and regulatory framework for Xenotransplantation.

NL EVD Internationaal China
http://www.evd.nl/home/landen/landenpagina/land.asp?land=chn NL EVD Internationaal is dé overheidsorganisatie die ondernemers inspireert en ondersteunt bij het waarmaken van hun internationale ambities. Hierbij gaat het om het aanbieden van informatie, financiering en netwerken. NL EVD Internationaal is onderdeel van Agentschap NL.

EXPLANATION OF THE STATE FOOD AND DRUG ADMINISTRATION WEBSITE

Introduction to the SFDA database

The State Food and Drug Administration (SFDA) is China's national regulatory authority in charge of food and drug ad-ministration. The SFDA oversees review and approval of clinical trial studies, drug registration, drug manufacturing and inspection, and licensing for drug importation.

According to the Drug Administration Law of the People's Republic of China, a new drug cannot be launched on the market until it obtains all the following approvals from the SFDA: approval for clinical trials (phases I, II, and III), New Drug Certificate, New Drug Registration Certificate, and Drug GMP (Good Manufacturing Practice) Certificate. Each stage is rigorously reviewed and evaluated by an expert panel authorized by the SFDA. The SFDA grants approval to merited applications.

Searching the SFDA database is probably the most reliable way to retrieve information on medicines that are currently in development or have been recently developed in China.

The SFDA website in English.

The English website shows brief information on the Chinese SFDA, including the introduction to SFDA, news reports, laws and regulations, and regulatory guide. However, for the updated database search and other more extensive information retrieval, the website in Chinese must be used.

The SFDA website in Chinese.

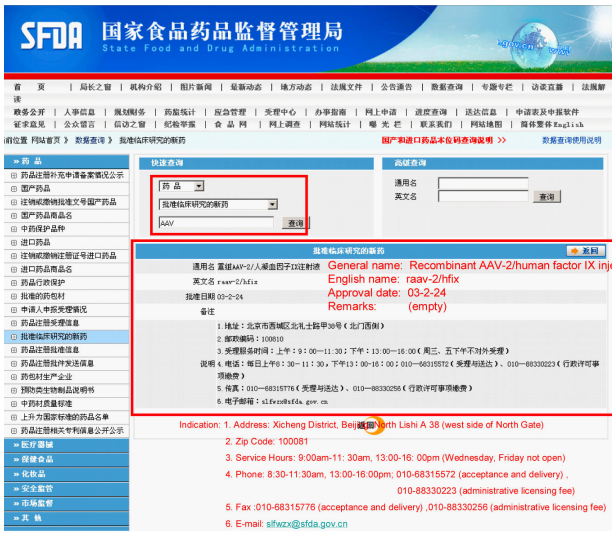
The Chinese website shows complex panels of all sorts of information related to the business of SFDA, for all types of clients/readers of the website. Among the 32 itmnes on the panel that locates on the top of the page, is the button of database search.

The SFDA database search function.

7 categories of items are listed on the database search page, including Medicine, Medical equipment, Health food, Cosmetics, Safety regulation, Market supervision, and Others. Each of these categories contains various subgroups. From a few steps of specification, the search page within "medicine" and "domestically developed" or "newly approved for clinical trial" is shown.

Search within " domestic medicine" or medicine newly approved for clinical trial".

For example, when seurching in"Medicine" + "newly approved for clinical trial" + keyword "AAV", the sesult shows 1 record.



The names and approval date are the only information. And sometimes the names are so abbreviated.. (see next page for example

Open this record. Translation see next page.

Both the Chinese and Englishs names are so abbreviated, so that one cannot find related reference from other resources for this item.

SFDA database in conclusion

- Usefulness: official and reliable resource for searching new medicines and their approval dates for clinical trials.
- Deficiencies:
  - lack of detailed information
  - lack of multiple keywords search
  - not available for non-Chinese readers

PATIENTS TRAVELING FROM THE NETHERLANDS TO CHINA FOR GENDICINE TREATMENT

[I] On the website of Cancer Therapy China (www.cancertherapychina.com), it is reported that five Dutch patients traveled from The Netherlands to China to undergo cancer treatment including Gendicine treatment. Anecdotal information is given below.

(1) A male patient (59 years) had a large liver tumor with metastases and abdominal pain after several unsuccessful chemotherapy regimens in the Netherlands. In January 2005, a combination of gene therapy, TACE, CIK-DC immunotherapy, and traditional Chinese medicine were utilized. Claimed results are: after two months of the treatment, including interventional therapy, the large tumor became mostly necrotic and shrank. Meanwhile, the enlarged lymph nodes in the groin and abdominal pain disappeared.

(2) In addition, a video is provided on a female patient from The Netherlands. She suffered breast cancer with liver metastases. She had received chemo in a local hospital and then came to China when no other treatment was available for her in The Netherlands, as stated on the above-mentioned website. Her condition was very bad when she arrived. She underwent gene therapy in Beijing. Her condition was claimed to improve greatly, with a remarkable shrinking of metastatic lesions.
[http://www.cancertherapychina.com/index.php?option=com_content&view=article&id=245:a-dutch-ladys-praise-to-bgwicc&catid=46:story&Itemid=108]

(3) A female patient (70 years) had metastatic colon cancer in liver and abdominal cavity plus abdominal pain with 3 months of survival expected by her Dutch doctor after surgical exploration of abdominal cavity. In July 2006, she underwent a combination of gene therapy, antiangiogenesis therapy, cancer stem cell targeting, mild chemotherapy, CIK-DC imunotherapy and traditional medicine. One month after treatment, patient's abdominal pain disappeared and serum CEA levels significantly dropped. Two months after the treatment, the liver metastases and all symptoms disappeared with the CEA level dropping to normal. Sixteen months after treatment the patient was still well. CT scan is shown on website.

(4) Furthermore, a female patient (55 years) diagnosed with lung adenocarcinoma with chest pain was given a combined therapy of chemotherapy, antiangiogenesis therapy, gene therapy, CIK-DC immunotherapy. Reported results: after two months of treatment (June 2007 – July 2007) the lung lesion shrunk significantly and all symptoms disappeared. 1 tumor disappeared and the other tumor shrank after 2 months treatment. CT scan is shown on website.

(5) A final story is on a female patient diagnosed with lung cancer. In The Netherlands she received a round of surgery, chemo, and radiation. Despite initial treatment, cancer slowly took over control. They heard about the Beijing cent from a friend who went there a while ago. The patient has gone through CIK, cryosurgery, and the Chinese medicine. At first she was so weak so could barely walk, also fever, coughing etc. After 2 months of treatment she's getting stronger and her cancer is under control, according to her partner.

[II] On the website of Great Wall International Cancer Center (Beijing, China, http://genetherapyhospitals.com/genetherapy.html), it is reported that three Dutch patients traveled from The Netherlands to China to undergo cancer treatment including Gendicine treatment. Anecdotal information is given below. The 3 patients are the same as given in the previous website (#1, #3 and #4).

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10. APPENDIX

Xenotransplantation

The shortage of donor organs is an urgent medical need that has been difficult to solve in countries all around the world, including China. Relief could be provided when xenotransplantation becomes a viable option. However, two important issues need to be addressed first, namely the immunological barriers and preventing the transmission of animal viruses to man. In order to overcome the immunological barriers, genetically engineered pigs have been generated, such as the currently available pigs expressing human complement inhibitors or lacking the expression of α-Gal epitopes, to prevent hyperacute xenograft rejection. Still, the issue of controlling delayed xenograft rejection or acute vascular rejection needs to be resolved. The safety concerns regarding potential transmission of infectious agents are focused on studies of porcine endogenous retroviruses (PERV), which are encoded in the germ line DNA, as well as exogenous viruses, especially four families of herpes viruses. Three classes of PERV have been identified (PERV-A, -B and -C). In contrast to PERV-C, PERV-A and PERV-B can infect human cells in vitro. However, recently in vivo recombination has been observed between PERV-A and PERV-C which can produce a human-tropic recombinant virus. Still, no evidence of human infection with any PERV, even with the recombinant PERV-A/C virus, has been reported. To eradicate the transmission risk of these types of viruses, specific knock outs of endogenous retroviruses or short interfering RNAs specific for PERV sequences have been proposed.

The exogenous viruses that have been studied are porcine cytomegalovirus (PCMV) and porcine lymphotropic virus -1, -2 and -3 (PLHV-1, -2 and -3). PCMV activation has been reported in pig-to-primate xenografts, causing clinical disease in the xenotransplanted organ and the detection of viral DNA in primate tissues. Still, it does not appear to cause invasive disease in transplanted primates. Furthermore, PCMV could be effectively excluded from source pigs by early weaning. Of the three PLHV viruses identified, only PLHV-1 is associated with a lymphoproliferetive syndrome similar to post-transplantation lymphoproliferative disease following allogenic bone marrow transplantation in swine, but such a disorder has not been observed in pig-to-primate xenotransplantation.

Concerns have been raised that some of the strategies aimed at minimizing xenograft rejection may increase the risk of zoonoses. In genetically engineered pig lines expressing human complement regulatory proteins, pig viruses may adapt to infect humans once porcine organs have been transplanted. Immune suppression and tolerance induction may exacerbate the risk of infection from otherwise non-infectious or latent animal pathogens. Finding the balance between maximizing the engraftment and minimizing the risk of cross-species transmission of viruses will be the key issue when entering the clinic. More detailed information can be found at the Xenome project website (http://www.xenome.eu/new_start.aspx).