Information destined for the public

Company: Transgene S.A.

Trial title: "**Clinical trials of non-specific immunotherapy by interleukin 2 expressed by a recombinant adenovirus**"

European notification number: **B/BE/03/B3**

The voluntary dispersion of genetically modified organisms (GMO) is strictly controlled in Europe by Directive 2001/18/EC of March 12, 2001 that replaced Directive 90/220/EEC, and in Belgium by a new Royal Decree « regulating the voluntary dispersion of genetically modified organisms in the environment as well as their marketing, and of products containing them » replacing the Royal Decree of December 18, 1998. The transposition process is currently under way.

In order to guarantee the risk-free use of GMO, the clauses of the above-mentioned Royal Decree stipulate that it is prohibited to market products without prior authorization from the competent Ministry. Granting an authorization depends on a thorough evaluation of the biosafety of the planned use, carried out by the Biosafety Council composed of several Scientific Committees including independent experts from Belgian universities or government institutes.

In order to obtain authorization from the competent Ministry, Transgene with headquarters at Strasbourg, France, has deposited an authorization application with the competent authority. Based on the decision of the Biosafety Council, the competent Ministry can authorize Transgene to conduct experiments with the genetically modified product TG1024 (Adenovirus IL2) according to the procedures described in application **B/BE/03/B3**.

The work will be conducted in the Medical Oncology Department of Professor Thierry VELU at the Erasmus Hospital in Brussels. It is planned to start experimentation in the first quarter of 2004 and to terminate in December 2004.

Table of contents

1 GENERAL INFORMATION

1.1 Description of the genetically modified microorganism (GMM)

The product TG1024, called Ad-IL2, is a suspension of a genetically modified type 5 adenovirus containing the gene for human interleukin 2.
This adenovirus is only slightly pathogenic for humans, causing only harmless respiratory problems {Takafuji, Gaydos, et al. 1979 #34660}. It has been genetically modified to eliminate its replication capacity and thus its already low pathogenicity, and to introduce a gene enabling the virus to produce the protein interleukin 2 (IL2), a protein naturally expressed in humans and having immuno-stimulating and anti-tumour properties.
The principle involves the direct injection of the product TG1024 in the tumour of patients to enable it to locally produce the protein IL2. The protein can then exert its anti-tumour effect by locally and remotely stimulating the immune system, at the same time as avoiding the toxic effects generally observed with IL2 administered alone intravenously or subcutaneously, often the reason for interrupting the treatment.

1.2 Type and aim of the planned trial

The trial whose code is TG1024.01 is a phase I/II clinical trial whose principal purpose is to determine the safety of repeated intra-tumour injections of the product TG1024, and to assess the maximal tolerated dose (MTD) in patients presenting a solid tumour (including melanomas) at an advanced stage. This initial phase of the trial has so far been assessed by treating five cohorts of three patients each, i.e. a total of 15 patients, with five increasing doses.
The MTD was determined on this basis. Four additional cohorts of three patients each , i.e. a total of 12 patients, should lead to the sequential determination of the intensification of TG1024 injection frequencies (cohorts 6 and 7 every 2 weeks, and cohorts 8 and 9 one injection per week), with the possibility of combining TG1024 with Dacarbazine (DTIC) chemotherapy (cohorts 7 and 9, administration of DTIC every 4 weeks).
Once the most promising treatment schedule in terms of safety and efficacy will have been established on the basis of cohorts 1 to 9, 12 additional patients will be treated according to this therapeutic protocol in order to assess the anti-tumour effect.
This trial will also lead to the determination of the biological effects and immune responses to the repeated intra-tumour injections of TG1024, as well as any possible long-term effects.
The second phase of the trial involves patients presenting a recently diagnosed melanoma and having received no prior systemic treatment.
The trial has been under way in Switzerland for several months and the patients recruited in Belgium will be included in the trial starting with cohort 7.

2 RESEARCH AND DEVELOPMENT ACTIVITIES

In spite of intense research, the poor long-term prognosis of patients with a metastatic cancer requires continued efforts. This is why new research orientations have been adopted, in particular immunotherapy and gene therapy.

The aim of immunotherapy in the treatment of cancers is to stimulate the immune system to recognize differences between tumour cells and normal cells in order to reject the cancer. Among results obtained, the most interesting have been obtained by treatments with specific cytokines that have occasionally led to the longest persistent responses {Baniel, Grauss, et al. 1998 #2020}{Bubenik 1990 #4600}.

The cytokine interleukin 2 is a powerful activator of cytotoxic functions (NK killer cells) and of immune system memory (T lymphocytes). The recombinant protein (synthetic) IL2 has been tested and its efficacy demonstrated in tumours of kidney cancer and melanomas with a 20 to 25% response rate {Rosenberg, Aebersold, et al. 1990 #30640}. The severe toxic effects associated with the systemic administration of IL2 have made this cytokine a good candidate for gene therapy. This is because the intra-tumour administration of the gene expressing IL2 limits the toxic effects of the protein in the bloodstream, at the same time as maintaining a locally high expression inside the tumour.

Gene therapy enables a given gene to be delivered to a precise location, followed by its local expression. Different types of vectors have been used to accomplish this, including adenoviruses that have a well known safety profile {Graham & Prevec 1992 #1970}{Schwartz, Togo, et al. 1974 #32380}.
Transgene has developed its adenoviral vector over several generations of the product, tested in several phase I trials that have shown the good safety of the products and the absence of significant adverse effects {Tursz, LeCesne, et al. 1996 #35820}. TG1024 enables a better expression of IL2 than older generation vectors, provides higher safety, and better anti-tumour responses have been observed with animal model tumours.

Animal experimentation has shown that administration of TG1024 in tumours led to their rejection in 33% to 65% of the cases, with increased survival {Slos, De Meyer, et al. 2001 #33130}. In addition, the combination of TG1024 injections with radiotherapy has given positive results in another animal model of tumours {Hillman, Slos, et al. 2003 #16130}. It was shown that the number of immune cells specific and toxic for the tumours treated increased after TG1024 treatment, confirming its immunological effect {Slos, De Meyer, et al. 2001 #33130}.

The toxicity of TG1024 has been studied in rats and cats. In the former, no major toxic effects were observed after intravenous or subcutaneous administrations. The repeated subcutaneous administration of a high dose of TG1024 to cats (equivalent to $3x10^{12}$ vp in humans) caused the death the animals 5 to 10 days after the first injection, attributed to the toxic effect of IL2. At an intermediate dose (equivalent to $3x10^{11}$ vp in humans), toxicity was reduced but still present, with the death of 2 animals among 6. Animals treated with a low dose (equivalent to $3x10^{10}$ vp in humans) exhibited several signs such as a slight fever, but overall the treatment was well accepted. Physiological particularities of cats make this species sensitive to the administration of viruses, which could explain the observed toxic effects {Warner 1996 #37430}{Winkler 1988 #38560}.

Based on these results, trials in humans were started with a low dose of $3x10^{8}$ vp, i.e. 100 times lower than the dose well accepted by cats, with a slow dose augmentation. A phase I trial was thus conducted in patients with metastatic melanoma or another type of solid tumour. The purpose of the trial was to determine the safety of repeated intra-tumour injections of TG1024 and to determine the Maximal Tolerated Dose (MTD). The maximal dose of TG1024 planned in the present trial is $3x10^{11}$ vp, about the same as the maximal dose administered (10^{9} pfu) in clinical trials with previous generation products having good safety.

The first part of this trial, TG1024.01, was conducted in Switzerland. At the present time, 25 patients have been treated with TG1024. Analysis has shown the good safety of the product administered every three weeks up to the dose of $3x10^{11}$ vp. The protocol of the trial under way was amended to extend it to phase I/II in order to increase the rhythm of TG1024 injections (every 2 weeks, then every week) and to assess safety and efficacy of combining

with a standard chemotherapy (dacarbazine) in melanoma patients. About 25 additional patients should be treated in this second part of the trial.

3 BENEFITS

As stated above and in spite of intense research, metastatic melanoma remains a disease with a poor prognosis and thus requires the development of new treatments. This has oriented research in new directions, in particular immunotherapy and gene therapy.

The purpose of immunotherapy in the treatment of cancers is to stimulate the immune system to recognize the difference between tumour cells and normal cells in order to reject the cancer. Among the results observed, the most interesting have been obtained by treatments with specific cytokines that have occasionally led to longer persistent responses {Baniel, Grauss, et al. 1998 #2020}{Bubenik 1990 #4600}.
The cytokine Interleukin 2 (IL2) is a powerful activator of cytotoxic functions (NK killer cells) and of immune system memory (T lymphocytes). The recombinant protein IL2 is marketed under the name of Proleukin® for the treatment of kidney cancer (renal carcinoma) and that of metastatic melanoma, with response rates of 20 to 25% {Rosenberg, Lotze, et al. 1989 #30660}. The systemic (intravenous or subcutaneous) administration of IL2, however, causes severe toxic effects, making it a good candidate for gene therapy. The intra-tumour administration of the gene expressing IL2 via an adenovirus limits the toxic effects of the protein in the bloodstream, at the same time as maintaining a locally high expression inside the tumour, enabling it to exert its anti-tumour effect

4 RISKS

Wild-type adenovirus causes harmless respiratory infections in humans and animals {Takafuji, Gaydos, et al. 1979 #34660}. This genetically modified virus has lost its pathogenic character as well as its capacity to propagate in human and animal cells, making it very safe in comparison to the risk of spread. It remains capable of having the cell it infect produce the protein whose gene it carries (interleukin 2) and to induce an immune response. The adenovirus is localised in the nucleus of the infected cell where it remains in the form of an episome with no known risk of inclusion in the genome of the infected cell {Horwitz 1990 #2170}. Since the recombinant adenovirus has lost it replication capacity, viral DNA is naturally eliminated when the infected cell dies.
The principal known side effects resulting from the administration of adenoviral vectors are flu type symptoms, increased hepatic enzymes and a decrease in platelet counts. The occurrence of these effects depends on the route of injection and the dose administered {Clayman, El-Naggar, et al. 1998 #6800}{Gahθry-Sθgard, Molinier-Frenkel, et al. 1997 #12490}{Stewart, Lassam, et al. 1999 #34110}{Liebert 2002 #22310}{Stephenson 2001 #33950}.

The principal known side effects resulting from the systemic administration of IL2 (Proleukin®) are low blood pressure, renal and hepatic toxicity, respiratory difficulties (dyspnea), fever, nausea, vomiting, loss of weight, erythema and skin rash, and anaemia. All these effects are reversible when treatment is stopped {Sleijfer, Janssen, et al. 1992 #33150}{Stein, Malkovska, et al. 1991 #34000}.

Up to the present, the principal side effects associated with the intra-tumour administration of TG1024 reported by patients are fatigue, erythema and pain at the injection site, fever, chills, nausea and vomiting, loss of appetite, headaches and dizziness. Fatigue, redness,

inflammation and discomfort at the injection site have also been reported with older generations of this type of vector.

5 CONFINEMENT, CONTROL AND MONITORING MEASURES

5.1 Control of the use and/or dispersion of the GMM or the gene

Conditions of storage and use:
TG1024 is a frozen preparation that must be stored at a temperature equal to or less than -70°C in a locked freezer under the responsibility of the investigator and/or pharmacist. Ampoules are distributed only after written authorisation by the principal investigator to specifically designated staff members.

Preparation for administration:
The trial product will be diluted with the TG0004 formulation buffer to obtain the desired concentration. The viral suspension to inject will be prepared in a laminar flow hood just before administration. A preparation protocol containing detailed instructions for the mixture will be provided by Transgene to the trial pharmacist/investigator.

The viral suspension will be administered by intra-tumour injection in the primary or secondary tumour with a 1 ml syringe.

Monitoring patients:
Blood samples will be taken from patients before and then 1 and 2 hours after the first two injections, and 24 h after the first injection in patient cohorts Nos. 1 to 7 in order to monitor potential viral spread. This will be analysed by polymerase chain reaction (PCR) (search for the presence of viral DNA) with the option of culture (search for infectious viral particles).

Data currently available show the presence of viral DNA in the blood of patients, measured with specific tests (PCR), 1 to 2 hours after injections but rarely and only at low levels 24 hours after TG1024 injection.

Complete kinetics of viral dispersion in patients cohorts 8 and 9 are planned for 3 patients of each cohort by the detection of viral particles in samples of blood, urine, faeces and throat swabs, before and during the four days following TG1024 injections on days 1 and 15. Virus monitoring and the other laboratory tests will not be conducted in the following 18 patients suivants is order to reduce patient monitoring.

5.2 Genetic stability of the GMM

Tests are conducted on each batch of the product destined for clinical trials in order to guarantee the integrity and functionality of the recombinant adenovirus genome.
The expression and functionality of interleukin 2 are also tested on each batch.

Recombinant adenovirus particles are produced in specific cells of the laboratory, PERC6. The recombinant adenovirus genome was specifically designed so that there are no regions homologous with the genome of PERC6 production cells. As a result, the risk of generating viruses with replication potential is unlikely. This parameter is also tested on each batch of product.

In addition, regions of the recombinant adenovirus genome indispensable for its replication (regions E1 and E3) have been deleted. It cannot replicate in the natural environment and thus requires the specific cells of the laboratory. The recombinant adenovirus does not replicate in humans and other mammals and no genetic instability is expected after administration to patients.

In spite of this, it cannot be ruled out that the recombinant adenovirus of TG1024 can exchange its genetic material during coinfection of the same human cell by a wild-type adenovirus and thus reacquire a replication capacity. The probability of occurrence of this event is extremely low and would involve only a limited number of viral particles which would be rapidly eliminated by the immune system and thus would have no effects on health.

5.3 Destruction of material containing the GMM

A detailed procedure for the preparation of the product will be furnished to the personnel involved in product preparation in the hospital departments in which patients will be treated by TG1024. A technical data sheet describing the procedure for injection, the conditions for eliminating waste and steps to take in case TG1024 is accidentally dispersed, will be placed in patient rooms. All waste arising from use of the product will be stored in a specific closed container that will be decontaminated with standard hospital procedures for infectious material.

5.4 Training requirements

At the time of the initiation visit organised by the trial applicant (sponsor) with the medical staff, all personnel participating in the clinical trial, physicians, nurses, pharmacist, will be informed in detail of the objectives of the clinical trial and its protocol, as well as the nature of the product to be handled, any risks related to the product, handling procedures to follow and steps to take in case of an accidental dispersion of the product. All these recommendations are also described in a synoptic document on product information entitled « Investigator Brochure » and in the technical data sheets remitted to the personnel participating in the trial.

5.5 Emergency situations

Throughout the treatment, patients receiving TG1024 will be monitored by the medical staff in terms of biological and clinical parameters at each weekly injection of TG1024 they will receive. An unexpected event occurring can thus be detected rapidly, and immediately managed on case-by-case basis.
Concerning handling the product in the Erasmus Hospital, recommendations will be furnished by the applicant for the following situations:
. In case of accidental dispersion of the product (ampoules broken), all contaminated must be treated according to standards hospital disinfection procedures.
. In case of a wound inflicted by a contaminated needle, the wound will be immediately disinfected with hydrogen peroxide and covered with sterile gauze. The person will consult with the physician responsible for the clinical trial in order to receive surveillance recommendations and will be monitored for at least 2 weeks.
. In case of contamination of the skin, there will be local disinfection with hydrogen peroxide and the surface of the contaminated skin will be thoroughly washed with soap and water.
. In case of ocular contamination, abundant rinsing with water will be followed by an ophthalmology examination as soon as possible.
. If the product is accidentally ingested, it is recommended not to induce vomiting and to immediately contact either the physician responsible for the trial or another physician. The person will be monitored for at least 2 weeks.

5.6 Responsibilities of the applicant

The authorisation that the competent Ministry may grant to the applicant will stipulate that he is responsible for any harm to human or animal health and that of the environment that could be caused by dispersion.

5.7 Inspection by public authorities

Inspectors will be empowered to verify if the applicant's trials are compliant with the conditions of the authorisation and to investigate any shortcomings. If shortcomings or fraudulent procedures are identified, the applicant will be open to sanctions.

5.8 Report of activities

At the end of the clinical trial, a report of activities drafted by the applicant will be remitted to the competent authority. This report will include not less than the following data:

- the site and period of use,
- the precise nature of the GMM really used,
- the aim(s) of the trial,
- measures taken to avoid involuntary dispersion of the transgenic material,
- if applicable, measures taken to protect the subject (patient/animal) during administration of the trial medication containing the GMM,
- if applicable, measures taken to protect the friends and family of the treated patient,
- measures taken to protect persons having handled the material containing the GMM,
- methods used for the destruction of unused or contaminated material,
- results of the trial,
- a summary of data concerning monitoring of GMM dispersion by the patient/animal,
- a summary of data concerning monitoring of the presence of the GMM of recombinant DNA in the environment.

6 REFERENCES

7 GLOSSARY

Ad	Adenovirus
Systemic administration	Intravenous or subcutaneous administration
DTIC	Dacarbazine
IL2	Interleukin 2
GMM	Genetically modified microorganism
GMO	Genetically modified organism
Pfu	Plaque Forming Unit
Vp	Viral particle

vp and pfu are units for measuring the number of viruses

8 CONTACT

If you have any comments concerning this public dossier or our activities, or if you wish to obtain additional information, you can contact the address found below.
You can also consult the summary of notification (SNIF) on the European Commission Research Web site (http://gmoinfo.jrc.it/). Comments can be sent to the Commission via this Web site.

8.1 Applicant

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