Comments and Concerns

on the application made by Prof. Glenda Gray

in respect of

HIV vaccine clinical trial of genetically modified organism (MRKAd5 HIV-1 gag/pol/nef)

by

African Centre for Biosafety

Submitted to

The Registrar: Genetically Modified Organisms Act
National Department of Agriculture
Private Bag X 973
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on

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ACKNOWLEDGEMENTS

The African Centre for Biosafety would like to thank several scientists, including Dr Michael Antoniou, Department of Medical and Molecular Genetics, King's College London School of Medicine, Guy's Hospital, London and Dr Veljko Velijkovic, Centre for Multidisciplinary Research and Engineering, Institute of Nuclear Sciences VINCA, Belgrade, Yugoslavia for their assistance with this submission.

Summary of concerns

There are a number of significant concerns pertaining to the application submitted to the National Department of Agriculture for approval of a HIV vaccine clinical trial of genetically modified organism (MRKAd5 HIV-1 gag/pol/nef) by Prof. Glenda Gray of the Perinatal Research Unit, Chris Hani Baragwanath Hospital. These relate to:

- the use of adenoviruses
- aspects of the risk assessment
- the definition of risk
- elements of the application, including lack of access to the Prior Informed Consent component

There are also a number of unanswered questions related to the health of the vaccinees as well as to the creation of new recombinant viruses and non-target effects.

1. Context

It should be noted that this submission is written in the context of a country that is devastated by HIV-AIDS at every level. That solutions are needed for this is without question. However, the use of a contested technology such as genetic engineering / genetic modification is disputed.

2. Application details

Applicant:	Professor Glenda Gray, Director of the Perinatal HIV Research Unit, Chris Hani Baragwanath Hospital
Application for:	HIV vaccine clinical trial of genetically modified organism (MRKAd5 HIV-1 gag/pol/nef)
Title:	A Phase IIB test-of-concept study to evaluate the safety and efficacy of a 3-dose regimen of the Merck Adenovirus serotype 5 HIV-1 gag/pol/nef vaccine in HIV-1-uninfected adults in South Africa.
Vaccine provider:	Merck & Co. Inc (Whitehouse Station, NJ, USA)

3. Access to information

The African Centre for Biosafety (ACB) has, in the past, made comment to the Department of Agriculture on applications relating to genetically modified food and agricultural crops. This application for a clinical trial of a GM vaccine is the first medical application that ACB has commented on. The lack of information in the public domain became quickly evident. Brief details were obtained from the press release informing the public of the application, but further information was not available. To comment effectively on an application, and therefore for the public participation process to work, it is important to have as much relevant information as possible. Although ACB is a South African public interest organisation, the applicant from a South African public interest research group, and the 3000 participants

planned for the trial will be people living in South Africa, detailed information was not easily obtained. Details of the process of obtaining access to information on the application follows:

- Brief details were obtained from the press release informing the public of the application in the Cape Times (26 April 2006).

- Further detail was sought from Prof. Glenda Gray, the applicant, who although willing to give access to the application, needed permission from the supplier of the vaccine, Merck & Co. Inc. (Whitehouse Station, NJ, USA) and the National Institutes of Health (NIH, Maryland, USA). This permission was not forthcoming.

- An application was then made to the Department of Agriculture on 11 May 2006, under the Promotion of Access to Information Act (PAIA), for the application, supporting risk assessment data and related documents. On 16 May 2006, a 53 page document was released.

- On 17 May 2006, a request was made to the applicant for a further extension of 30 days to comment on the application. This was agreed to.

- Details of the Prior Informed Consent (PIC) of the participants were not contained in the 53 page document and so a request for this was made to Prof. Gray. However, because it formed part of the protocol, Prof. Gray said it could not be released and ACB was referred to the Medicines Control Council (MCC), Prof. Cleaton-Jones of the University of the Witwatersrand Ethics Committee and Mr Ian Burns of the University of the Witwatersrand Biosafety committee. (The application besides going through the Department of Agriculture and the GMO Act also needs approval in South Africa from the MCC and the relevant ethics and biosafety committees).

- After some discussion, Prof. Patrick Arbuthnot, chairperson of the University of the Witwatersrand Biosafety Committee, directed us to the University of the Witwatersrand Ethics Committee because the request was for information relating to Prior Informed Consent. A letter has been sent to Prof. Ames Dhaia, Chairperson of the Ethics Committee, asking if it is possible for the Ethics Committee to make available this information or to facilitate a process whereby this information could be made available.

- Trying to obtain PIC information from the MCC was difficult - on 31 May 2006, the MCC was contacted for this information, but they needed a protocol number to follow up the request. On 2 June 2006, Prof. Gray was contacted for the number, but the response was that the MCC hadn't given her one. On 13 June 2006, the MCC was contacted again and Ms Nolunto Qodi, Acting Head of Clinical Trials, re-affirmed that a protocol number was needed. On 14 June 2006, Prof. Gray's office was contacted, but as she was out of the country it was suggested that another person in the office be contacted to assist. This was done, but a response was only received on 23 June when, on requesting confirmation from Prof. Gray that there was a definite "Prior Informed Consent" component to the application, the necessary details were sent – the protocol number as well as the MCC Database tracking number and MCC reference number. However, this delay meant that there was insufficient time to lodge a PAIA application with the MCC and get feedback before the 24 June 2006 deadline of this submission.

- A request was made to Prof. Gray on 14 June 2006, to grant a further extension to comment until 10 July 2006, in order to include comment on the PIC, but this request was not granted.

4. Background to the application

4.1 The vaccine.

The vaccine is a modified, transgenic Adenovirus type 5 (Ad5) vector vaccine. The vaccine is trivalent, i.e. the HIV *gag*, *pol* and *nef* genes have been cloned into separate vector genomes, and all 3 vectors are given in the same vaccine dose. The Ad5 genomes have been made replication deficient by deleting the E1 region of the genome and replacing it with the transgene expression cassette. Within the expression cassettes the transgenes are governed by the CMV (human cytomegalovirus) immediate early (IE) promoter and the BGH (bovine growth hormone) polyadenylation signal sequence. Infectious, recombinant Ad5 virus particles are generated by transfection of the respective genome constructs into E1-expressing PER.C6 cells for rescue. The resulting Ad5 vectors were then cloned, and single clones were selected and amplified for vaccine production.

4.2 The vaccine trial.

The safety and efficacy of a 3-dose regimen of the vaccine will be evaluated in a Phase IIB test-of-concept study. The study will be carried out with healthy, HIV-1-uninfected (18 to 35 years old) participants. At least 40% of them should have baseline Ad5 titers (must mean anti-Ad5 antibody titers?) below 200. The number of participants will be 3000, 1500 receiving vaccine and 1500 a placebo. The trivalent vaccine will be administered by intramuscular injection into the deltoid muscle.

It should be emphasized that the trial is carried out with *healthy individuals*.

5. Comments and concerns

There is a significant difference between applications such as this medical application and applications for genetically modified (GM) food and other agricultural crops - the restrictions and safety issues and checks around this application are extensive and detailed. It is correct that they should be, but similar stringency should be applied in South Africa to GM food and agricultural crops.

From some points of view it can be stated that the risk assessment is carefully planned and interpreted. It does, however, leave a number of unanswered questions related to the health of the vaccinees as well as the creation of new recombinant viruses and non-target effects. There are concerns with the use of adenoviruses, aspects of the risk assessment, the definition of risk and with elements of the application. Not having access to the Prior Informed Consent component of the application, has also meant that it is impossible to assess whether the vaccinees will be adequately informed about the full nature of the clinical trial that they are going to participate in. These concerns are dealt with in more detail below.

5.1 Adenoviruses.

Members of the family *Adenoviridae* are ubiquitous. In addition to the 51 human types characterized so far, all mammalian, avian and reptilian species studied are hosting adenoviruses. Studies of animal adenoviruses are mostly scanty, and it may be postulated with confidence that many of them are yet to be found.

While adenoviruses generally infect mucosal epithelium, different serotypes differ in their tissue-specificity, and infection ultimately results in cell destruction. Pathogenicity varies according to group and type, and organ specificity and disease patterns appear to cluster within particular subgroups of adenoviruses. In general, adenoviruses may persist in their hosts, and may be detected and shed months after primary exposure.

It has been generally assumed that adenoviruses are host-specific, and that they do not readily cross host species barriers. This assumption was not based on much experimental or ecological evidence, and exceptions to the rule are known. During the last years a number of examples of potential inter-species adenovirus infections have been published, although rather modest investments seem to have been made to elucidate such events.

In the context of replication incompetent adenovirus vectors, the present state of knowledge opens up for *worst-case scenarios* related to i) Infections in non-target individuals/species and ii) New viral strains created by recombinations between vectors and naturally occurring, replication competent viral strains.

5.2 Risk issues

Worst case scenario
Annex II of the EU Directive 2001/18/EC describes the principles for risk assessment (RA) relevant for the present case. The RA, according to a recent report, “does not necessarily have to be based on data of the scenario that is expected to occur, but can also be based on a worst case scenario. In an RA this is a common way to go forward, if there is scientific uncertainty about the expected scenario.”

A possible worst case scenario is that a systemically spread replication deficient Ad5 vector is rescued by a concomitantly infecting wild type human adenovirus. This might lead to shedding of infectious adenovirus(es) from the vaccinated individual(s). The characteristics of such viral strains are per definition unpredictable. They might be subject not only to complementation of the E1 region of the Ad5 vector DNAs, but would also depend on the actual wild type adenovirus strain(s) present and on additional recombination events, homologous as well as non-homologous, for other parts of the adenovirus genome. It is now generally assumed that in an evolutionary context these are the mechanisms by which new adenovirus serotypes have been, and will continue to be, created.

An extrapolation of this scenario would include the following. Replication competent adenovirus(s) are shed from the vaccinated individuals. These individuals have contact with pet or other domestic animals (e.g. dogs, cats, cattle, sheep, horses, pigs). The animals are infected with their own wild type adenoviruses. The originally transferred human virus strains, or novel strains appearing as a result of recombinations with animal strains, are shed and transmitted to wild life animal species. These in their turn harbour their own adenovirus strains etc. Disease may occur at any stage in this “relay”, and finally novel adenoviruses with unexpected characteristics may return from wildlife to domestic animals and humans.
The probability of all these events may be very low, but the risks may, according to the risk definition be high.

Definition of risk
The term “risk” is very often confused with “probability”, and hence used erroneously. Risk is defined as the probability that a certain event will take place *multiplied by* the

consequences arising *if* it takes place. This means that an event carrying a very low probability may represent a tremendous risk if the consequences are serious and/or irreversible.

With regard to the development and commercialization of genetically engineered nucleic acids, organisms and viruses we often are neither able to define probability of unintended events nor the consequences of them. Hence, the present state of ignorance may make scientifically based risk assessments impossible. This calls for invoking the "Precautionary principle".

Conclusions of the Applicant's Risk Assessment
The applicant has, in some respects, delivered a solid and insightful report. A number of potential harms, hazards and issues of scientific uncertainty and ignorance have been discussed. In their final conclusions they do, however, generally characterize all potential risks as "negligible". It is not clearly described how these conclusions were reached, and many of them are not backed up by scientific literature or results from their own experiments.

The impression is that the applicant has confused "probability" and "risk". It is *assumed* that the probabilities are low, and hence the risks are also considered low – to the level of being "negligible".

A more thorough description of the scientific knowledge and data supporting the conclusions is needed. *Specific concerns and questions about the application follow in 5.3 and need to be addressed*.

5.3 Specific concerns and unanswered questions

i) In the applicant's evaluation it is assumed that human serotypes do not normally infect other species. What is the scientific basis for this assumption?

ii) It is stated that long term persistence of the Ad5 vector requires integration into the host genome. Integration frequency is about 1 per 10^5 plaque forming units in exponentially growing cultures of primary human cells. Has the risk evaluation of the applicant ruled out this possibility?
a) Comment: Experiences with other integrating viruses demonstrate a vast efficiency variance according to the cell types used and the experimental design.

iii) Is it conceivable that the other transgenic elements of the expression cassette represent any risks, e.g. by recombination with HIV and cytomegaloviruses in the human organism, or BGH-like genomic sequences ?

iv) The stability of the Ad5 vector has not been considered to be different from wild type adenoviruses, due to the size of the insert. DNA insertions into other DNA virus genomes are prone to destabilize the genome. Are there any particular reasons that adenoviruses should be exempted from this?

v) **Testing of RCA (replication competent adenoviruses) shedding**
a) Comment: this cannot be considered precise data. The figures will most certainly vary with the test system and recipient cell cultures employed.
b) Question: Should testing in a panel of relevant cell cultures not be a requirement for clinical trials?

vi) Post trial monitoring has been found unnecessary by the applicant

a) Question: In light of the arguments presented should marketing not be made dependent on an adequate post trial monitoring plan?

b) Question: Should not post trial monitoring also require monitoring of effects on domestic and wildlife animals?

vii) Tumorigenicity studies in newborn rats (pp 17-18)

a) Why was the observation period restricted to 6 months?

b) Why was only one of the vectors tested, while the trivalent vaccine was used for the toxicology assessments?

c) Why were the observed cases of tumors considered unrelated to the vector?

d) Which methods were used to exclude genomic vector integration and e.g. CMV promoter mediated overexpression of an endogenous oncogene?

viii) Toxicology and safety assessment studies (pp. 25-27)

a) "The vector DNA was "predominantly found in…." Where else was it found?

b) "The vast majority of the vector remained extrachromosomal". Where was the minority located?

c) Which methods were used to detect chromosomal integration? What were the target detection limits of the tests?

d) Why, and based on which criteria, were all symptoms and pathological findings judged "within acceptable limits for a vaccine", "no treatment-related effects", "of minimal toxicological significance" etc.? What are the criteria for these judgments and assumptions? Where are the data backing them up?

e) Why were no efforts of viral recovery reported for the animal experiments?

f) Why have no double infection experiments, vaccine plus wild type adenovirus(es) been performed?

ix) Adenoviruses and RA

In view of the general description of "Adenoviruses": Why have all questions and risk issues related to environmental effects been answered as "N/A"? The whole rationale for these conclusions is based on assumptions and lack of relevant studies.

x) Use of the vaccine in the future?

Dr Michael Antoniou[1] has expressed the concern that to date trials and tests have only been conducted on normal subjects who are HIV/AIDS negative; i.e. those that are not immune compromised. He notes that normally adenoviruses, including the first generation Ad-vector that is to be used in these trials are highly "immunogenic"; that is they elicit a strong immune response, so the infection is cleared quite quickly, in people with normal immune system function. In the context of the proposed trial he states that this is a mixed blessing. In all likelihood the subjects in the trial will develop a good immune response against the Ad-vector itself, as well as any therapeutic effect against HIV. This is acknowledged as a possibility and certainly experience from other trials involving the same first generation Ad-vector this is a well-documented and common occurrence. From the point of view of the trial this is not good as any anti-Ad-vector immunity will compromise the effect of the second and third follow up vaccinations that are

[1] Department of Medical and Molecular Genetics, King's College London School of Medicine, Guy's Hospital, London.

planned at 28 and 182 days after the first dose. From a safety point of view this is obviously good as it adds an extra barrier to the spread of virus from the injected subject to others. This is all well and good in normal subjects. What happens if this vaccine was in future administered to people who already have AIDS and are immune compromised? There would be little or no anti-Ad-vector immunity. And if Ad-vector and normal adenovirus end up in the same AIDS immune compromised person, there would be no innate immunity check on the replication and spread of the normal and Ad-vector virus.

Although this will not apply to the proposed trials since the Ad-vector will only be administered to healthy HIV-1 uninfected participants, it should be asked how it is anticipated using this vaccine in the future? Will only healthy HIV-1 uninfected people be eligible to receive it? Will the SA health service spend huge sums of money and time screening people first to see if they are already HIV and/or AIDS positive before deciding whether or not to give this Ad-vector vaccine? Would they not just vaccinate all people, at least within the highest risk groups in the country?

5.4 Prior Informed Consent

Of particular importance in this application is the Prior Informed Consent component - to see how the trial is described to the volunteers and how the matter of genetic modification is dealt with (not to access information of possible commercial significance). For example, are the vaccinees aware that they will be vaccinated with a genetically modified organism? Is the information adequately translated into the mother tongue of the vaccinees?

It is unfortunate that access to this component could not be obtained timeously. It is within the powers of the applicant, the vaccine provider and the sponsors to make available this data and why not? It should not be something that is hidden – it should be easily available to comment on within the framework of the GMO legislation – not only something that should be dealt with by ethics committees and the MCC. Is it co-incidental that the vaccine provider and sponsors are located in the USA, who unlike SA is not a signatory to the Cartagena Protocol on Biosafety? Perhaps the standards of biosafety are too weak in the USA?

6. Recommendations to the Executive Council (EC)

A few recommendations are made in order to facilitate easier participation by interested parties in the future:

- For the roles and links between the various regulatory bodies (EC, MCC, ethics and biosafety committees) in GMO medical applications to be made clear.

- For the EC to urgently re-assess the information that is regarded as Confidential Business Information in an application. For example, information relating to Prior Informed Consent (PIC) should not fall under this category – it is a critical component of the application and is something that interested parties should be able to access.

- For applicants and their commercial partners to be fully briefed on the rights of interested parties to have access to relevant information and to interrogate that information.

7. Conclusion

Considering the above – the concerns with the use of adenoviruses, the gaps in the risk assessment, the many specific queries and unsettled issues – it is incumbent to repeat the call for the "Precautionary principle" to be applied and for a moratorium on clinical trials of AIDS vaccines based on adenoviral vectors.

Submission copied to:

- Prof. Ames Dhaia, Chairperson of the University of the Witwatersrand Human Research Ethics Committee
- Prof. Patrick Arbuthnot, Chairperson of the University of the Witwatersrand Biosafety Committee
- Ms Noluntu Qodi, Acting Head of the Clinical Trials Unit, Medicines Control Council
- Zackie Achmat, Chairperson of the Treatment Action Campaign (TAC)