
Pathogen reduction in blood products





To: The Minister of Health,
Welfare and Sport

Re : presentation of advisory report on "Pathogen Reduction in Blood Products"
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Dear Minister,

The statutory remit of the Health Council consists not only of issuing advisory reports requested by Ministers or State Secretaries, but also of proactively drawing the attention of those officials to issues and trends that are of relevance to government policy. The Health Council does this in separate publications that have an alerting function. It is with this purpose in mind that I hereby present the 'alerting' advisory report "Pathogen Reduction in Blood Products". In preparing the report, the Health Council's Blood Working Group was acting in the capacity of a committee. The document has been reviewed by the Standing Committees on Medicine and Infection & Immunity.

In this advisory report the Health Council outlines the current level of knowledge on inactivation techniques that are potentially interesting methods for further reducing the risk of infections being transmitted via blood transfusion. At this point in time, however, the Health Council is unable to recommend introduction of the inactivation techniques since there is still too little published clinical research and there are still too many uncertainties and questions.

In 2004 the Health Council plans to weigh the added value of new techniques against the costs of introducing them in a report entitled "The Safety of Blood". This assessment will also be used to take stock of the inactivation techniques in the event that more data becomes available in the future.

Yours sincerely,

Prof. dr JA Knottnerus

Pathogen reduction in blood products

to:

the Minister of Health, Welfare and Sport

No. 2003/16E, The Hague, 28 August 2003

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Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.



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Executive summary

Inactivation of micro-organisms in blood

Work has been under way for some time in the field of blood transfusion medicine on the development of techniques for disinfecting donated blood. If it were to be 100% successful, this approach would offer the major advantage of removing all pathogenic micro-organisms from donor blood, even those for which the blood currently is not – or cannot be – tested. Theoretically, the introduction of these so-called pathogen inactivation techniques would mean that the selection of donors and the testing of the blood for the presence of micro-organisms would become matters of secondary importance.

Various research groups and pharmaceutical companies are working on inactivation techniques based on compounds that penetrate genetic material (DNA or RNA) of micro-organisms and then bind inextricably with it. As a result, the micro-organisms are no longer able to proliferate and perish. Because the compounds used can also bind to human genetic material, laboratory research into the safety of the technique is particularly important.

The inactivation techniques for platelets, transfusion of which accounts for around 20% of all transfusion procedures, are now so advanced that routine use is considered to be a possibility. Here the Blood Working Group of the Health Council of the Netherlands acts as committee and gives its opinion on the desirability of introducing inactivation techniques in the Netherlands. It bases this opinion on an inventory of the available scientific research.

Research results

The techniques developed have undergone various forms of testing in the laboratory. Inactivation experiments have been performed with viruses and, to a lesser extent, with bacteria and parasites. Inactivation of viruses usually results in a reduction of at least ten thousand-fold. In the case of extremely large quantities of micro-organisms, however, the inactivation techniques appear to result not in total inactivation but merely a numerical reduction. This limitation of the technique places question marks over the possibility of dispensing with other safety measures when introducing inactivation techniques.

Experiments with blood products such as platelets, plasma and red blood cells demonstrate that, although the products may well still conform to the relevant quality requirements, the treatment usually has a negative impact on quality.

The toxicological research conducted to date has largely focused on the question of whether the substances used to treat the blood products, which bind to DNA or RNA, still elicit harmful effects following completion of the procedure. The results of the research indicate that this is not the case. However, it is not clear what consequences exposure in the long term, or – in the case of patients receiving blood products on a regular basis – repeated exposure to small quantities, might have. Owing to the limitations of the research conducted to date, a definitive verdict on the safety of the techniques will probably only be possible following large-scale administration, as in the case of standard use in bloodbanks or hospitals – especially if the adverse effects occur only rarely.

There have been few publications to date on clinical research involving inactivation techniques. Most of the data is derived from two phase-III studies with Amotosalen, a compound based on a group of substances known as psoralens. The American SPRINT trial showed that patients who were given platelets treated with Amotosalen required 35% more blood transfusions than the patients who received platelets treated in the standard manner. In the European euroSPRITE trial, with a smaller number of patients, the patients who were given platelets treated with Amotosalen did not require statistically significant more transfusions than the patients given conventionally treated platelets. This research shows that there is no statistically significant difference between the “corrected count increment” (i.e. the increase in the number of platelets following correction for the patient's body surface area) in patients given Amotosalen-treated platelets one hour after transfusion and the increase recorded in patients given the conventionally treated platelets. Twenty-four hours after transfusion, however, the corrected count increment in the patients receiving Amotosalen-treated platelets was lower than that recorded in the patients given the conventionally treated platelets. In the research with compounds other than Amotosalen, too, there is evidence to suggest that the treatment of platelets or red blood cells results in loss or reduced survival of the product.

Opinion on introduction

The committee advises against the introduction of inactivation techniques for micro-organisms in blood products at this point in time.

The committee arrives at this decision in view of the paucity of published clinical research. There is, at present, only one publication on phase-III research and there is no published clinical research with the transmission of micro-organisms via blood transfusion as its endpoint. Research with such an endpoint would, in fact, require vast numbers of patients in view of the fact that transmission already occurs very rarely even with the existing safety measures.

In the Netherlands the administration of blood products has, over time, become a progressively safer medical procedure as far as the transmission of micro-organisms is concerned. The residual risks of blood transfusions for which inactivation techniques would be beneficial are: virus infections in the period between the penetration of the virus and the moment at which its presence becomes detectable by means of tests (the so-called “window” phase); infections with micro-organisms for which testing is not yet performed; bacterial infections caused by contaminated platelets; and parasitic infections. A number of these risks have already been reduced through the adoption of measures such as the testing of platelets for bacterial contamination by means of culturing. Inactivation has no effect on the other residual risks, such as the mixing-up of blood products and transfusion-related acute lung injury. These risks will assume still greater prominence if the provisional data on the reduced effectiveness of the treated blood products is confirmed. This could, after all, mean that patients will be administered blood products more frequently than at present.

The committee anticipates that research designed to determine the complete chain of events that occurs during blood transfusion – such as, for example, the recently commenced programme of the Association for Transfusion Reactions in Patients (TRIP) – will provide quantitative data concerning the residual risks of blood transfusion in the Netherlands. This data may pave the way for a modification of the position adopted here with regard to the status of inactivation techniques.

Introduction

The administration of a blood product is a therapeutic procedure whose safety cannot be completely guaranteed (and probably never will be). When in the western world blood is collected and blood products are prepared, a series of measures are taken in order to ensure that the end product is as safe as possible. These measures range from the selection and screening of donors to storing the final product under the correct conditions. In addition, the donor blood is examined for the presence of various micro-organisms. Separate tests are currently used in the Netherlands for, among others, hepatitis-C virus and HIV. If the outcome of a test indicates the presence of the micro-organism, the blood is excluded from further processing.

Work has been under way for some time on the development of techniques for disinfecting donor blood. If it were to be 100% successful, this approach would offer the major advantage of removing all pathogenic micro-organisms from donor blood, even those for which the blood currently is not, or cannot be, tested. Theoretically, the introduction of these so-called inactivation techniques would mean that the selection of donors and the testing of donated blood for the presence of micro-organisms would become matters of secondary importance.

Various research groups and pharmaceutical companies are working on inactivation techniques based on compounds that penetrate the genetic material (DNA or RNA) of micro-organisms and then bind inextricably with it. As a result, the micro-organisms are no longer able to proliferate, and they perish. Human platelets and adult red blood cells do not contain any DNA or RNA, apart from mitochondria. White blood cells, on the other hand, do contain DNA and RNA and are therefore inactivated when these tech-

niques are employed. This could mean that graft-versus-host disease (GVHD), which sometimes occurs after blood transfusion and is caused by white blood cells, will no longer occur when the inactivation techniques are used.

Because the compounds used can also bind to human genetic material, laboratory research into the safety of the technique is particularly important. The results produced to date by this laboratory research were sufficiently positive to allow for clinical research. The initial results from these studies are now being published in the scientific journals. The techniques for the transfusion of platelets, which account for around 20% of all transfusions, are now so advanced that routine use in bloodbanks is considered to be a possibility. A decision will probably need to be made within a few years in the Netherlands with regard to the introduction of inactivation techniques in the supply of blood.

Since the supply of blood in the Netherlands falls within the responsibility of the Sanquin Blood Supply Foundation, the Minister of Health, Welfare and Sport has requested the Health Council to pay special attention to the scientific developments in the field of blood transfusion medicine. The Blood Working Group, established for this purpose by the Health Council, indicates which developments and potential problems are relevant to policy-making.

In this ‘alerting’ advisory report, the Blood Working Group (henceforth referred to as “the Committee”) gives an overview of the current level of knowledge on inactivation of pathogens in blood products. Because it has emerged that the use of inactivation techniques does not result in total inactivation in the case of large quantities of (pathogenic) micro-organisms, but serves only to reduce the number of micro-organisms, the Committee speaks in this advisory report of pathogen reduction. The Committee identifies those points that, in its opinion, need to be considered when making the decision on introduction, such as the effectiveness of the inactivation process, the undesirable effects of the technique, and the functionality of the treated blood products. The Committee concludes the advisory report by offering its opinion on introduction. The membership of the Committee is given in Annex A.

In chapter 2 the Committee provides an overview of the current safety measures and the residual risks associated with blood transfusion. It also examines the differences between (short shelf-life) cellular blood products, such as platelets, red blood cells and plasma, and the (long shelf-life) plasma products, such as coagulation factors. In chapters 3 and 4 the Committee names the inactivation techniques that have been developed and discusses the research results that have been achieved with these techniques. In chapter 5 the Committee summarises the points that, in its opinion, are of importance

when making a decision on the introduction of the technique. Finally, in chapter 6 the Committee states its position on the introduction of the technique.

Present situation

In this chapter the Committee gives an overview of the safety measures for, and the residual risks of, receiving blood products. Here the Committee also examines the differences between cellular blood products and plasma products, because these differences may have a bearing on whether or not inactivation techniques are adopted.

2.1 Existing safety measures

The first part of the series of safety measures is the same for all blood products. Donors are selected and screened before the blood is collected. The collected blood is then examined for the presence of hepatitis-B virus, hepatitis-C virus, HIV, human T-cell leukaemia/lymphoma virus (HTLV) and *Treponema pallidum*, the bacterium responsible for causing syphilis. These examinations have long made use of either tests that detect the presence of the micro-organisms themselves (the so-called antigen tests) or tests for the antibodies that are produced as a result of the infection. More recent innovations are more sensitive tests based on the detection of DNA or RNA from the micro-organism. These tests provide a positive result within a shorter time after penetration by the micro-organism than is the case with the antigen and antibody tests. The advantage of this early detection is that it reduces the period (known as the "window" phase) between penetration of the micro-organism and the moment at which tests can detect its presence. The final safety measure that was adopted for all products some years ago is the removal of the white blood cells (leukodepletion).

The second part of the chain of safety measures consists largely of measures that are specific to each particular product. Since the end of 2001 in the Netherlands, for example, platelets have been tested for bacterial contamination by post-production culturing. There are various procedures during the preparation of plasma products that are designed to further reduce any viruses that may be present. The techniques employed in this connection cannot be applied to cellular blood products. Finally, the blood products are stored under ideal conditions – that vary from one product to another – up until the moment of administration.

2.2 Residual risks

Data from abroad

The adopted safety measures have brought about a marked decrease in the risk of the investigated micro-organisms being transmitted via blood products. Research shows that the risk of viral transmission, in particular, has decreased sharply. The risk of infection with hepatitis-B virus via blood transfusion is estimated at around 1:200,000, the risk of HIV and hepatitis-C virus at 1:1,000,000, and the risk of HTLV lower still¹⁻³. Research tracking the complete chain of events that occurs during blood transfusion – the so-called haemovigilance programmes – shows that the mixing-up of blood products, bacterial infection by contaminated platelets, and transfusion reactions as transfusion-related acute lung injury (TRALI) and graft-versus-host disease (GVHD) pose (far) greater risks for the recipient of blood products than transmission of the viruses for which testing is currently carried out⁴⁻⁹. In the United States, for example, it is thought that the administration of contaminated platelets could be responsible for causing as many as a thousand cases of sepsis per year⁹.

Transfusion reactions are adverse reactions experienced by the recipient of the blood product. These reactions usually occur where the recipient of the blood product forms (or has formed in the past) antibodies to components of the administered cells¹⁰. The reaction of the antibodies with the cells can have serious clinical consequences. In the case of TRALI, too, the reaction is presumed to be triggered by antibodies, though the precise underlying mechanism is not known¹¹. Unlike in the case of transfusion reactions, however, the antibodies that are involved in TRALI generally originate from the donor of the blood product¹². GVHD can occur where white blood cells from different individuals come into contact with one another – e.g. as a result of organ or bone-marrow transplantation – but also following blood transfusion. The donor's white blood cells recognise the cells of the recipient as being “foreign” and will, in general, trigger an immune reaction against these cells. This immune reaction can lead to GVHD, with sometimes serious clinical consequences¹³.

Transmission via blood transfusion has also been reported for viruses other than those for which testing is now routinely performed, such as cytomegalovirus, Parvovirus B19 and West Nile Virus^{10,14,15}. Cytomegalovirus and Parvovirus B19 infections only pose a risk for a proportion of blood product recipients. West Nile Virus is currently a particularly fast-growing problem in the United States. The US Food and Drug Administration (FDA) has proposed that blood banks question donors about symptoms that are typically associated with West Nile Virus infection¹⁶. It is not clear at this point in time whether the causative agent of Severe Acute Respiratory Syndrome (SARS) is also transmissible via blood transfusion.

Data from the Netherlands

The risk of micro-organisms such as HIV and hepatitis-C virus being transmitted via blood transfusion in the Netherlands is comparable with that in other western countries¹⁷.

Although it is anticipated that the screening of platelets for bacterial contamination will, indeed, bring about a fall in the incidence of sepsis in the recipient, it will not wholly eliminate this problem.

There have been no occurrences of GVHD following blood transfusion in the Netherlands in recent years. This is attributable not only to the routine removal of white blood cells from donor blood (so-called universal leukodepletion), but also to the irradiation of blood products destined for people at greater risk of GVHD (e.g. immunocompromised patients). Any remaining white blood cells are inactivated as a result of this, thereby preventing the occurrence of GVHD.

The Health Council has recently recommended that blood products intended for recipients at risk for Parvovirus B19 infection should also be screened for this virus¹⁸. This measure is now being implemented by the Sanquin Blood Supply Foundation ("Sanquin"), the organisation responsible for the manufacture of blood products in the Netherlands.

To date, two patients infected with West Nile Virus have been reported in this country^{19,20}. Both had recently visited an area with a high incidence of the virus. Sanquin asks donors about symptoms that could be suggestive of avian influenza or SARS.

2.3 New safety measures

In this advisory report the Committee concentrates on the inactivation of micro-organisms in blood products. However, there are also other developments in the area of safety measures. The majority of them involve an extension or optimisation of existing princi-

ples, such as the addition of a laboratory test for West Nile Virus or the treatment of the donor's skin with several disinfectants. Other developments are aimed at reducing contamination of donor blood with the donor's skin bacteria by not using the first 10-30 millilitres of donated blood for transfusion purposes. The initial results of research into this procedure do, indeed, indicate a lower percentage of contaminated donations^{21,22}.

2.4 Cellular blood products and plasma products

Cellular blood products differ from plasma products in a number of respects. In an earlier advisory report, the Health Council has already described the difference in the numbers of donors and recipients¹⁸. Cellular blood products are derived either from one or from a limited number of donors and are only administered to a limited number of recipients. Plasma products, on the other hand, are produced from so-called plasma pools (plasma donations that are collected from frequently very large numbers of donors) and are ultimately used in large numbers of patients.

Responsibility for the manufacture of both cellular blood products and plasma products in the Netherlands lies with Sanquin. In this country, therefore, the manufacture of both product groups is conducted on a non-profit basis and use is made of blood provided by unpaid donors. In other countries the manufacture of blood products often takes place under different conditions. As in the Netherlands, the cellular blood products are not manufactured for profit (and their provision therefore comes to be regarded more as a care service), whereas the manufacture of plasma products is commonly in the hands of commercially oriented pharmaceutical companies that quite often make use of paid plasma donors.

The Dutch Minister of Health, Welfare and Sport has declared her wish to provide for optimum, rather than maximum, safety with regard to the manufacture of cellular blood products²³. However, as far as the (international) trade in plasma products is concerned, there is a tendency to aim for an end product that offers maximum safety. This may possibly be influenced by the fact that there are greater risks of micro-organisms being transmitted where use is made of material obtained from paid donors²⁴.

Techniques for pathogen reduction

Methods of inactivating micro-organisms in blood products are being developed by various research groups and companies, sometimes on a collaborative basis. The techniques are usually geared towards just one type of blood product (platelets, plasma or red blood cells), or else they are individually tailored to suit each of these products on account of their differing characteristics and storage conditions. In this chapter the Committee describes three methods that have been developed by three companies.

3.1 Amotosalen and S-303

Cerus, in a joint venture with Baxter, is working on inactivation techniques based on so-called psoralens. These are chemical compounds that penetrate the DNA or RNA, with which, after further treatment, they form a covalent bond. This bond serves to prevent replication and transcription of DNA and RNA. Certain psoralens occur in nature, albeit in minute quantities. In the 1980s psoralens were used in the treatment of psoriasis and cutaneous T-cell lymphoma, a malignancy that manifests primarily in the skin^{25,26}.

Cerus/Baxter has developed Amotosalen (until recently used under the code name S-59) for platelets and plasma. After having penetrated the DNA or RNA, Amotosalen forms a covalent bond with the nucleotides as a result of irradiation with ultraviolet light²⁷. The remaining, unbound, Amotosalen is inactivated by the irradiation. The variant that has been developed for use in bloodbanks incorporates a compound that is attached to a carrier and absorbs the greater part of the residual Amotosalen and the photoproducts generated in these processes²⁸.

The characteristics of red blood cells make them unsuitable for methods that involve the use of ultraviolet light. Cerus/Baxter has therefore developed for red blood cells a molecule termed FRALE (frangible anchor-linked effector), which is used under the code name S-303^{29,30}. This molecule consists of three parts: the active component that binds to nucleic acid in DNA or RNA, an "anchor moiety" that is instrumental in transporting the complex to DNA or RNA, and a linking moiety. The complex is activated by the change in acidity that occurs following addition to the red blood cells. In the course of this process, the linking moiety decomposes and the anchor moiety is inactivated. Here too, the residual chemicals are absorbed.

3.2 Riboflavin

As far as the development of inactivation techniques is concerned, Gambro BCT has opted for an approach resembling that of Cerus/Baxter. The naturally occurring B2 vitamin, riboflavin, has been chosen as the DNA-binding or RNA-binding agent. Following irradiation with visible light, riboflavin fractures the nucleic acid chain of DNA or RNA, which results in inactivation³¹. Treatment with riboflavin can be applied to platelets, plasma and red blood cells.

3.3 Inactine

For red blood cells, Vitex has collaborated with Pall Biomedical in developing so-called Inactine, a compound that consists of two components^{32,33}. One component binds to nucleic acid, while the other forms a covalent bond with guanine, one of the bases from which DNA and RNA are formed. This bond also results in inactivation. The remaining Inactine is removed by washing the red blood cells.

Research findings

4.1 Laboratory research

4.1.1 *Inactivation of micro-organisms*

Most of the data on the efficacy of the various compounds in the laboratory are derived from research with viruses. There is considerable variation in the volume of research into each particular method for the inactivation of bacteria. Experiments with parasites have only been described for *Trypanosoma cruzi*, the causative agent of Chagas' disease³⁴. The data have in some cases been published in peer reviewed journals, but more often they have either been presented by the manufacturer during meetings or else they are available on request from the manufacturer. Summaries of the results of inactivation experiments have been based both on published data and on data obtained from the manufacturer³⁴.

The results of the experiments are usually expressed in terms of a reduction in the number of micro-organisms added and are presented on a logarithmic scale. A reduction of 3 ¹⁰log will therefore mean that the number of micro-organisms is a thousand times lower after processing than at the outset. There is a consensus regarding the production of plasma products that a reduction of 6 ¹⁰log to 8 ¹⁰log in the number of micro-organisms – achieved through a succession of measures – is sufficient for a safe product. In the case of cellular blood products, total removal of the micro-organisms is, in fact, the only acceptable option.

An overview of the results obtained shows that the reduction was usually at least 4¹⁰log (i.e. ten thousand-fold)³⁴. The inactivation techniques appear not to lead to total inactivation when there are large numbers of micro-organisms, but only to a decrease in the number of micro-organisms. Thus, regulators in the US prefer the term "pathogen reduction" to "pathogen inactivation"³⁴.

4.1.2 *In-vitro function of treated products*

Platelets

There is no generally accepted standard test for laboratory research into platelet function³⁴. Researchers make use of more general tests such as analysis of acidity or the number of abnormal platelets.

When compared with platelets treated in the standard manner, treatment of platelets with Amotosalen resulted in lower acidity, a rise in oxygen content, a lower platelet count and a higher percentage of irregular platelets³⁵. These changes mainly occurred at the end of the 5-day storage period and even then the platelets still satisfied the used quality requirements. Treatment of platelets with riboflavin likewise resulted in lower acidity and a rise in the oxygen content³¹. No reports were made on the platelet count and the percentage of irregular platelets.

Plasma

The quality of plasma has likewise been examined for treatment with Amotosalen³⁶ and riboflavin³¹. Treatment resulted in a variable decline of between 0% and 30% in the activity of the examined plasma proteins. According to Goodrich, such a reduction is well within the accepted norm³¹.

Red blood cells

The quality of red blood cells has been examined following treatment with Inactine³³. When compared with red blood cells treated in the standard manner, the Inactine-treated red blood cells exhibited a lower energy level. According to the authors, the red blood cells satisfied the relevant quality requirements. Treatment with Inactine had no effect either on haemolysis or on the expression of the surface antigens of the red blood cells.

4.1.3 Toxicity

Much attention has been paid during development of the methods to the removal of the chemicals that remain after binding with the DNA or RNA has taken place. If the inactivation techniques were to be introduced, an active component would in any case be added to a blood product, with this component being selected for its capacity to penetrate genetic material. This component does not only bind to the DNA or RNA of micro-organisms; it is also able to bind to the DNA or RNA of the blood product recipient. Despite the incorporation of removal steps in the procedure and the inactivation of this component, a great deal of laboratory research has been conducted into the possibility of toxicity.

In research on acute and chronic toxicity in dogs, Amotosalen exhibited toxic effects at doses of 40 mg and 30 mg per kg bodyweight, respectively. Doses of 30 mg and 25 mg per kg bodyweight, respectively, produced no toxic effects. According to the author, 40 mg Amotosalen per kg bodyweight is 40,000 times the expected amount of free Amotosalen that can find its way into the body of a blood product recipient²⁸. In monkeys, transfusion of treated platelets resulted in ventricular extrasystoles in 3 of the 12 animals studied. Based on subsequent, more specific research, the author concluded that the treated platelets were not responsible for the extrasystoles²⁸. Extrasystoles are said to frequently occur spontaneously in monkeys³⁷.

In a phototoxicity study where rats were irradiated with UV light following administration of Amotosalen, irradiation resulted in skin abnormalities after a single dose of 10 mg Amotosalen per kg bodyweight. In female rats (but not in males) these abnormalities were also observed at a dose of 1 mg Amotosalen per kg bodyweight (1,000 times the expected amount of free Amotosalen). According to the author, comparable skin changes in irradiated but untreated rats indicate that this particular rat species is highly susceptible to UV radiation²⁸.

No abnormalities were discovered in the course of this study on genotoxicity, reproductive toxicity, carcinogenicity and irritation of the blood vessels^{28,38}. In the research designed to evaluate the carcinogenicity of Amotosalen, experiments have also been conducted with test animals that are highly susceptible to the development of malignant tumours (heterozygotic p53 transgenic mice)³⁸.

4.1.4 Graft-versus-host disease

The compounds developed for the inactivation of micro-organisms also bind to the DNA or RNA of human white blood cells (among others). Treatment of blood products could therefore serve to reduce the incidence of GVHD. In experimental animals, treatment of

white blood cells with Amotosalen does, indeed, serve to prevent GVHD³⁹. Laboratory research with human white blood cells shows that treatment with Inactine prevents the immune reaction that underlies GVHD⁴⁰.

4.2 Clinical research

The number of publications on clinical research with the inactivation techniques is relatively small. Only for Amotosalen have data been published from randomised research into the treatment of haemato-oncological patients (phase III study). The publications about the techniques used with red blood cells concern research into efficacy (phase II study) in healthy volunteers. A substantial proportion of the publications are abstracts of papers or posters from scientific meetings. The Committee discusses the data on clinical research for each system and summarises the status of this research in the table.

4.2.1 Amotosalen

Platelets

The effect of treating platelets with Amotosalen has been investigated in two comparable phase III studies. The European euroSPRITE trial is a controlled, randomised, double-blind study in patients with thrombocytopenia following treatment with cytostatics. The endpoint in the trial was the increase in the platelet count, either or not corrected for the patient's body surface area (the so-called “(corrected) count increment”). The platelets used in this study were obtained from the buffy coat, the fraction of the (centrifuged) blood in which the majority of the platelets are found. The results of this trial have previously appeared in abstract form⁴¹ and were published quite recently⁴².

This research showed that for the first eight transfusions there was no statistically significant difference in the corrected count increment one hour after transfusion between patients given Amotosalen-treated platelets and patients given the conventionally treated platelets. The corrected count increment at 24 hours after transfusion (once again for the first eight transfusions) in patients receiving Amotosalen-treated platelets was lower than that in patients given the conventionally treated platelets. Analysis of *all* transfusions revealed no statistically significant difference (neither after one hour nor after 24 hours) between the count increment for the Amotosalen-treated platelets and the conventionally treated platelets. No difference was discovered between the two types of platelets with regard to the haemostatic effect of the transfusions, the undesirable effects of the transfusions or the incidence of bleeding.

The American counterpart of the euroSPRITE trial – the SPRINT trial – has thus far only been published as an abstract⁴³. The endpoint in the SPRINT trial was the inci-

dence of bleeding, and the platelets were obtained through apheresis (a technique whereby the platelets, but not the other blood components, are harvested from the donor). The trial group was larger than in the euroSPRITE study and the patients were in a poorer clinical condition, given that more than 75% of them had undergone an allogenic stem cell transplantation.

In the SPRINT trial, the Amotosalen-treated platelets proved just as effective in combating bleeding as the conventionally treated platelets. However, the administration of Amotosalen-treated platelets resulted in a smaller count increment and corrected count increment than the administration of the conventionally treated platelets. The lower count increment meant that the patient group that was given Amotosalen-treated platelets received 35% more blood transfusions⁴³.

Plasma

The first publication on the use of Amotosalen in plasma appeared recently. This is a phase II study in which the amount of coagulation factor VII in the blood of healthy volunteers was artificially lowered⁴⁴. The increase in factor VII achieved through administration of the Amotosalen-treated plasma was no different from the increase following administration of the conventionally treated plasma⁴⁴. The first results of phase III research in a limited number of patients with hereditary abnormalities of blood coagulation have been published in abstract form⁴⁵. The authors concluded that the administration of Amotosalen-treated plasma appears to be an effective treatment method.

4.2.2 S-303

The results published to date for S-303 are from phase II research⁴⁶. This study involving healthy volunteers revealed no difference in recovery and survival between red blood cells that had been treated with S-303 and red blood cells treated according to the standard method.

4.2.3 *Inactine*

Two abstracts have been published on the results of phase II research with Inactine^{47,48}. In both studies, the effect of treatment with Inactine and storage for a given period was analysed. After 28 days of storage no differences were observed, as far as haemolysis and survival were concerned, between the Inactine-treated red blood cells and the conventionally treated red blood cells⁴⁷. Extending the period of storage from 28 to 42 days produced no difference in terms of haemolysis, but the survival of Inactine-treated cells after 42 days of storage was lower than that of the conventionally treated cells⁴⁸.

Table Status of research with inactivation techniques.

technique	blood product		
	platelets	plasma	red blood cells
Amotosalen	III ^{42,43}	II ⁴⁴ , III ⁴⁵	n/a
S-303	n/a	n/a	II ⁴⁶
Inactine	n/a	n/a	II ^{47,48}

n/a: not applicable

Points to consider during decision-making

Here the Committee gives an overview of the topics which it believes warrant consideration when decisions are made on whether or not inactivation techniques should be introduced in the Netherlands. The Committee discusses these topics and identifies their consequences for the decision-making.

5.1 Laboratory research

5.1.1 *Inactivation of micro-organisms*

For the majority of micro-organisms, the research into inactivation reveals a reduction of at least 4^{10} log. A number of comments need to be made on these results. For example, some viruses, bacteria and parasites are able to invade the blood cells of the host. The inactivation of intracellular micro-organisms can prove more problematic than under laboratory conditions, where the micro-organisms are not present in cells.

In the case of viruses and parasites in particular, the scope for laboratory experiments is limited by the fact that the organisms are difficult (and sometimes completely impossible) to grow in the laboratory. The implications of this situation are discussed separately for each of these groups of micro-organisms.

Viruses

It is sometimes impossible to perform inactivation experiments with pathogenic viruses because these viruses cannot be grown in the laboratory. Researchers must then resort to model viruses. Although these may well be selected for their similarities to the pathogenic viruses, they are not identical to them. The results of the inactivation experiments performed in the laboratory are also limited by the maximum concentration of the cultured virus. In the case of a virus with a maximum yield of 10^5 viral particles per millilitre, it will never be possible to demonstrate inactivation in excess of $5 \log^{10}$ in a laboratory. It is therefore unclear whether the extremely large numbers of viral particles that are encountered in some viral infections can be completely inactivated using the present techniques. Infections with Parvovirus B19, for example, can give rise to 10^{14} viral particles per millilitre of blood⁴⁹.

This limitation raises questions over the feasibility of abandoning other safety measures when introducing inactivation techniques. Moreover, it is not clear whether the inactivation techniques can prevent an infection with an as yet unknown virus ("the next virus") for which no other safety measures are in place.

Bacteria

The majority of bacterial species can be cultured in the laboratory, which means that research into the effectiveness of inactivation techniques for bacteria is relatively straightforward. However, there is evidence to suggest that inactivation is influenced by the bacterial life cycle. For example, inactivation of the so-called spore-forming bacteria appears to be problematic⁵⁰. It is possible that intracellular bacteria may be more difficult to inactivate than bacteria that circulate freely in the bloodstream.

Parasites

Laboratory research with parasites is often problematic, not only on account of the culturing problems discussed earlier, but also because parasites have a relatively complex life cycle. In the course of this cycle, the organism will manifest in several different forms, including intracellular and extracellular life-stages. The inactivation techniques need to be effective during each of these stages. In the Netherlands, in fact, transmission of parasites via blood transfusion is relatively uncommon.

5.1.2 *In-vitro* function of treated products

In the laboratory, the treated blood products conform to the relevant quality requirements. However, the published findings of experiments with platelets, plasma and red blood cells do show that treatment has an adverse effect on the quality of the blood products.

5.1.3 *Toxicological* research

The toxicological research carried out to date with inactivation techniques does not point to any harmful effects. These studies have focused primarily on the question of whether the DNA-binding or RNA-binding substances administered to the blood products still cause any harmful effects following completion of the procedure. The research findings indicate that this does not apply in the case of the expected quantities. However, it is unclear what might be the consequences of exposure in the long term, or – in the case of patients receiving blood products on a regular basis – repeated exposure to small quantities.

Owing to the limitations of the research conducted to date, a definitive verdict on the safety of the techniques will probably only be possible following large scale administration, as in the case of standard use in bloodbanks or hospitals. This is especially the case if the adverse effects occur only rarely³⁴.

5.2 **Neoantigenicity**

In August 2002 the FDA organised a two-day workshop on inactivation techniques. Among the topics addressed during this meeting was neoantigenicity⁵¹. Neoantigens are produced where drugs (or their metabolites) form such a bond with endogenous substances (freely circulating proteins such as enzymes, but also cell-associated structures) that these are recognised by the immune system as “foreign”. This recognition may mark the beginning of an immune reaction against the endogenous substance, which can give rise to serious medical conditions. The occurrence of disease as a result of the formation of neoantigens has been reported after various interactions (such as the interaction between enzymes and drugs⁵², and that between red blood cells and antidepressants⁵³ or anti-inflammatory drugs⁵⁴).

The question of neoantigens is of relevance in relation to inactivation techniques, since it is known that some of the compounds used in these processes also bind to other endogenous substances besides DNA or RNA²⁸. In research into the toxicity of Amotosalen and in the clinical research with Amotosalen, no evidence has been found to sug-

gest that neoantigens are formed^{28,42}. However, it is precisely because the formation of neoantigens may possibly be relatively rare that this phenomenon may indeed have remained unobserved during the present phase of research.

5.3 Clinical research

The clinical research findings published to date have attracted critical comment. AuBuchon, in particular, comments on the results published so far⁵⁵. In his opinion, there is evidence in each of these studies to suggest that the treatment of platelets or red blood cells using the newly developed methods results in loss or reduced survival. Some of these consequences had already been highlighted by the authors of the relevant studies.

In the Amotosalen trials^{41,43} the treated platelets exhibit shorter survival than the control platelets. The patients who were administered the treated platelets in the American study consequently received more transfusions than did the patients from the control group. In the trial with S-303⁴⁶ the loss of S-303-treated red blood cells was greater than the loss of control red blood cells. Finally, in the study with Inactine⁴⁸ the survival of the Inactine-treated red blood cells after 42 days of storage was lower than that of the control red blood cells.

The criticism leveled at the research has no bearing on the safety of the administered treated platelets or red blood cells. However, the other risks for the recipient – e.g. the mixing-up of blood products – could well come to play a greater role in the event of blood transfusions being administered more frequently. Furthermore, if the greater loss and reduced survival of the treated platelets or red blood cells were, in fact, to result in more frequent administration of a blood product to a patient, these factors will have repercussions on the costs of using the techniques.

The Committee is not aware of any clinical research on inactivation techniques that has the transmission of micro-organisms via blood transfusion as its endpoint. Indeed, the Committee realises that research with such an endpoint would require vast numbers of patients, since transmission already occurs very rarely even with the existing safety measures.

5.4 Risks following introduction of the inactivation techniques

The receipt of a blood product would still be associated with certain risks even after the possible introduction of inactivation techniques. Inactivation of micro-organisms will not mitigate the risk of a blood product mix-up, transfusion reaction or TRALI. Inactivation may well reduce the risk of bacterial infection as a result of contaminated platelets. In the Netherlands efforts are already being made to curb the incidence of these infec-

tions by post-production screening of platelets for the presence of bacteria by means of culturing. The risk of GVHD (already very remote) has been further reduced in this country through the introduction of universal leukodepletion. The irradiation of blood products that is now practised in connection with some patient groups could well be abandoned if the inactivation techniques were to be adopted^{39,40}.

If the existing screening tests for viruses are maintained, introduction of the inactivation techniques in the Netherlands would, at best, bring about a marginal reduction in the (already very small) risk of virus transmission. The question is: might this potential health benefit not be negated by the risks that could be associated with introduction? The Committee believes that this question can only be answered by means of careful postmarketing surveillance, for example through the above-mentioned haemovigilance programmes. Such a programme was also recently started in the Netherlands by the Association for Transfusion Reactions in Patients (TRIP)⁵⁶.

5.5 Costs

Owing to the differences between platelets and red blood cells, it is impossible to develop a single system whereby the technique can be applied to both products or to the blood directly after donation. Consequently, if the inactivation techniques were to be introduced, platelets (and plasma) would need to be treated with a different system than red blood cells. This would have repercussions both on the direct costs of equipment and materials, and on the indirect (logistical) costs.

To date the manufacturers have made no official announcements about the costs, but the expectation is that these will be considerable. Apart from what the manufacturer charges for the equipment and the material, the blood transfusion organisations will most likely also charge for the increased personnel costs and the costs of introducing the technique. The costs of treating a patient will escalate still further if the provisional data regarding the reduced effectiveness of the treated blood products are confirmed. This could, after all, mean that patients will be administered blood products more frequently than at present.

A number of years ago it was suggested that the introduction of inactivation techniques for red blood cells could result in some of the current safety tests being discontinued. Now this appears to be less likely, however, until it has been conclusively demonstrated that the techniques developed can bring about complete inactivation. Moreover, it is extremely rare in western blood transfusion medicine for safety tests to be abandoned once they have been introduced*.

* In Sweden it was decided in 1995 only to test new blood donors for HTLV⁵⁷. This decision was reached following a cost-effectiveness analysis of the screening of all donors over the course of one year.

A cost-effectiveness analysis would appear to be premature at this point in time, given the uncertainties over the costs and possible benefits of pathogen reduction in blood products.

5.6 Maximum or optimum safety

Where inactivation techniques have been shown to add value in terms of safety, their introduction might be consistent with the pursuit of maximum safety that has hitherto been customary in international blood transfusion medicine. According to this policy, any test that increases safety will be introduced, irrespective of the costs. However, it is questionable whether this goal of maximum safety can be maintained in the light of spiralling costs. The Minister of Health, Welfare and Sport in the Netherlands has declared her wish to provide for optimum, rather than maximum, safety²³. Optimum safety means seeking a balance between the health benefit that is afforded by a proposed measure and its costs.

The fact that the provision of blood in the Netherlands lies in the hands of a single organisation means that, as far as blood products are concerned, this country's policy with regard to safety measures is one of uniformity. Such uniformity does not exist at the international level. In principle, one country can introduce a safety measure while another country refrains from doing so. Decision-making in other European countries can be further complicated by the fact that the supply of blood lies in the hands of several organisations.

In order to achieve maximum uniformity in policy-making, European harmonisation would appear to be desirable when making decisions about the (possible) introduction of the inactivation techniques. This is particularly so since the international exchange of (and trade in) blood and blood products are resulting in ever-higher safety requirements. At this moment this is especially important in connection with plasma products, which are regarded as tradable goods. However, the possibility cannot be ruled out that in the future the cellular blood products will also increasingly come to be viewed from an economic perspective.

Opinion of the Committee

Inactivation techniques for micro-organisms in blood products are based on an elegant concept. By rendering pathogenic viruses, bacteria and parasites harmless or reducing their numbers (pathogen reduction), the risk of disease being transmitted via blood transfusion is further diminished. Inactivation techniques are, however, also the first techniques whereby a compound that reacts with DNA or RNA is added to a blood product. The Committee concludes the overview of research findings presented in this advisory report by giving its opinion on the introduction of the techniques.

The Committee advises against the introduction of inactivation techniques for micro-organisms in blood products at this point in time.

The Committee arrives at this decision largely in view of the paucity of published clinical research. At present, there is only one publication on phase III research. Moreover, there is no record of any clinical trial in which inactivation has been shown to reduce the risk of disease and infection being transmitted, and it is questionable whether such research will ever come about.

As far as the transmission of micro-organisms is concerned, the administration of blood products has, over time, become a progressively safer medical procedure following the adoption of various measures, both in the Netherlands and elsewhere in the western world. The advantage to be gained from introducing inactivation techniques is consequently minimal (additionally so because some of the residual risks are not influenced by inactivation). According to the Committee, the advantage of introducing these techniques could be further diminished by the possible undesirable effects of the com-

pounds involved, though little evidence has been produced to date of undesirable effects in the short term. Little or no data is available, however, on long-term undesirable effects.

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A The Committee

Annex

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