

REPLACED SHEETS	SG
☒ Description : paged ...1... to paged ...12...	
☒ Claim(s) : paged ...13... to paged ...14...	
☒ Drawing(s) : paged ...15... to paged ...16...	
☒ Abstract : paged ...17... to paged ...18...	
DATE OF RECEIPT	

1

The present invention relates to a composition for use in the treatment of tumours and the immunization of organisms against tumours.

It has been established very recently by various scientific teams that the localized injection, into organisms with a tumour, of syngenic tumour cells producing an interleukin could result in the rejection of this tumour by the organism.

This was demonstrated for Interleukin-2 by Bubenik et al. (Immunology Letters 19, 279-282, 1988; Immunology Letters, 23, 287-292, 1989) and confirmed in particular by Fearon et al. (Cell., 60, 397-403, 1990) and by Ley et al., (European Journal of Immunology 1991, 21: 851-854; Res. Immunol., 1990, 141: 855-863).

The authors of these articles mention that rejection is accompanied by memorization of the response. The animal is therefore vaccinated against subsequent development of the same type of tumour even if the latter is grafted onto a different site.

Syngenic cancer cells producing Interleukin-4 have also been tested with similar results, as reported by Golumbek (Science, 254, 713-716, 1991) and Tepper et al. (Cell, 57, 503-512, 1989) and also cells producing the tumour necrosis factor (TNF) as described by Blankenstein et al. (J. Exp. Med., 173, 1047-1052, 1991).

This Sheet Has Been Replaced



The systems described in these publications contain disadvantages for their application in man.

In all these publications the cells producing interleukin are cells from the individual or from a syngenic individual, which were modified to express interleukin.

For the treatment of humans, one disadvantage of this method is the risk that the cells expressing interleukin injected into the organism might continue to develop even after rejection of the tumour.

To remedy this drawback a method of treatment was tested which consists of injecting into the organisms allogeneic or xenogeneic cells expressing genes enabling them to produce in vivo one or more biologically active substances, such as Interleukin-2 (cf. patent application FR 91 14 119 dated November 15th, 1991 entitled: « Cell composition for the treatment of human or animal organisms »).

This method permits the transient treatment of the organism with these substances as the cells, on account of their immunological nature, are rejected by the organism.

This treatment was tested by injection in the vicinity of the tumour cells (LPB tumour) of Interleukin-2 producing allogeneic cells 9 days after inoculation with the tumour

This Sheet Has Been Replaced



cells. A beneficial effect was observed in the form of diminished tumour growth over several days.

This is a transient effect and, under the conditions used, does not always cause induction of immunity
5 memorization specific to the inoculated tumour cell. In animals treated in this way subsequent inoculation of the same tumour cell (Lewis tumour) can lead to tumour growth.

Moreover the effect observed, that is to say diminished tumour growth, is not always sufficient to cause
10 elimination in the entire tumourous mass.

It will be noted that, in all the experiments described in the state of the art the absence of tumourous growth is generally measured on a healthy animal, and very rarely on pre-established tumours.

15 GOLUMBEK et al. (1991) Science, 254, 713-716) and PORGADOR et al. ((1992) Cancer Res. 52: 3679-3686), for example, have injected a composition in both these cases for the purpose of treating a preestablished tumour, but at the time of treatment the preestablished tumour was neither
20 visible nor macroscopically detectable.

With a different approach TROJAN et al., (1992) Proc. Natl. Acad. Sci., USA 89, 4874-4878, (1993) Science, 259, 94-97) have modified the immunogenicity of a tumour in the rat (glioma) by transfecting the tumour cells with a vector
25 coding for a complementary DNA antisense to IGF 1 (Insulin-



like growth Factor 1). The authors mention that the injection of these modified cells results in the absence of tumorigenicity and in a remote effect on a preestablished tumour. Nevertheless this approach is extracted to tumour cells secreting IGF as autocrine growth factor and it requires the manipulation of each tumour cell to generate a specific immunity response.

Another proposal based on the infection of tumour cells with a retrovirus carrying the gene encoding the thymidine kinase of herpes was tested on two models. In the first model Culver et al. ((1992) Science, 256, 1550-1552) have developed a therapeutic approach in which a rat glioma (brain tumour) is injected with xenogeneic cells producing a retroviral vector into which was inserted the gene coding for the herpes thymidine kinase. After local production of TK^+ viral particles, which will infect the rapid growth cells (tumour cells), systemic treatment with Ganciclovir (Merck Index, reference 4262), induces massive regression of the pre-established tumours mass. However this type of treatment is not fully effective since, in the described experiment, complete macroscopic and microscopic tumours regression was observed in only eleven animals out of the fourteen treated. Also, a small number of tumour cells (4×10^4 cells) are injected and treatment (injection of fibroblast cells producing TK^+ viral particles) is carried



out at a very early stage (from Day 5 onwards) after inoculation of the tumour cells. The major disadvantage nevertheless remains the non-observance in this model of immunity memory vis-à-vis secondary inoculation of the same
5 tumour cells.

The second model develops a relatively similar approach to that mentioned above. Established macroscopic, hepatic tumours are treated by intratumourous injection of xenogeneic fibroblasts producing viral particles expressing
10 the TK gene of the herpes virus and selectively infecting the tumour cells. After a period of transduction of the TK gene in the tumour cells in vivo, most of the tumours mass is eliminated by treatment with Ganciclovir (GVC). However, the major disadvantages remain the same, namely the absence
15 of any guarantee regarding the full elimination of tumour cells and memorisation.

Both these approaches have recourse the xenogeneic cells capable of expressing viral particles able to transduce the TK gene in the tumour cells. The second
20 approach, compared with that developed by Culver et al., is more effective as it mimics a situation of hepatic metastases of primary colon cancer and it is applied to a well developed tumours site that is macroscopically visible.

This Sheet Has Been Replaced



Finally, a technique based on the combined use of electric impulses and local injections of allogeneic or xenogeneic cells secreting Interleukin-2 has recently been developed (MIR et al., (1992) Compte-Rendu de l'Académie
5 des Sciences de Paris, série III, 314, 539-544).

According to MIR et al. (Eur. J. Cancer 1991, 27, 68-72), electrochimiotherapy consists of locally injecting bleomycine and applying electric impulses in the vicinity of the tumour.

10 The combined use of these two methods potentialises the anti-tumours effect observed in each of the two methods used singly.

However, electrochimiotherapy has the major disadvantage, even in combined use with cells secreting
15 interleukin-2, of being easily applied only in tumours with at least one accessible site.

It therefore arises clearly from the state of the art analysed above that the methods described are not always effective, particularly against established tumours, and
20 that they do not systematically provide immunity memory specific to that tumour or only permit the local treatment of tumours.

The applicants therefore have sought to find a composition to obtain firstly the fast disappearance of the
25 tumour wherever it may be localised, and secondly the long-



term, specific immunisation of the organism against the treated tumour.

They subsequently found, in surprising manner, that with the combination of immunomodulator secretion means and
5 vectors leading specifically to the suicide of tumour cells, it is most often possible to obtain the rapid, definitive disappearance of the tumour and specific long-term immunization of the organism against this tumour.

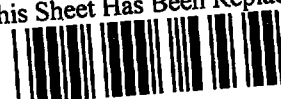
The object of the present invention therefore is a
10 composition for use in the treatment of tumours in human or animal organisms and to immunise them against this tumour, said composition comprising a synergistic association of:

- cells, viruses or bacteria transiently expressing in the organism at least one gene enabling them to produce in
15 vivo one or more immunomodulators, and

- viruses, or cells producing viruses, said viruses if possible preferably infecting dividing cells of the treated organism and carrying within their genome at least one gene whose expression in the dividing cells will cause their
20 destruction.

Advantageously, the composition such as described above comprises cells producing in vivo one or more immunomodulators and in addition producing viruses carrying within their genome at least one gene whose expression in
25 the dividing cells will cause their destruction.

This Sheet Has Been Replaced





The cells used in these compositions shall preferably be allogeneic or xenogeneic cells so that they may be eliminated from the treated organisms. They may themselves be infected by or be producers of viruses carrying within
5 their genome at least one gene whose expression in the dividing cells will cause their destruction such as defined above.

Said composition may thus comprise cells in which the virus used to infect the dividing cells of the organism is
10 responsible for the production of the immunomodulator.

The immunomodulators are advantageously Interleukin-2, Interleukin-4, Interleukin-7, the Tumour Necrosis Factor (TNF), Interferon-gamma, GM-(Granulocyte-Macrophage Colony Stimulating Factor), alone or in combination.

15 These immunomodulators may, in addition to the cells and bacteria mentioned above, be expressed from genes carried on the virus genome, such as retroviruses, Pox viruses (vaccine virus, Canary Pox), Adenoviruses and defective virus associated with Adenoviruses (AAV).

20 The bacteria may be intracellular bacteria producing an immunomodulator.

It will be noted that the immunomodulators may be brought by any means, in addition to cells, bacteria or viruses, enabling the release of immunomodulators in the
25 organism; one such means for example is a grafted micropump releasing immunomodulators in the organisms.



The viruses preferably infecting the dividing cells of the treated organism and carrying within their genome at least one gene whose expression in the dividing cells will cause their destruction, or lysis agents, are preferably
5 retroviruses, Pox viruses or Adenoviruses. These viruses will be all the more advantageous if they have the property of infecting or destroying preferably and for the most part the dividing cells of the organism.

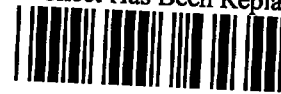
They may be brought by selected cells so that they may
10 be eliminated rapidly in the organism, particularly allo- or xenogeneic cells.

The gene whose expression in the dividing cells will cause their destruction is preferably the gene of a thymidine kinase or a cytosine deaminase. The destruction
15 of the cells carrying these genes shall be induced by supplying them respectively with Ganciclovir and 5-fluorocytidine.

The present invention also relates to the use of the above described composition for the manufacture of a
20 medicinal product for the treatment of tumours and cancers and for the immunization of the organism against these illnesses.

Advantageously all the elements of the composition are administered simultaneously. They may be given
25 simultaneously or independently.

This Sheet Has Been Replaced



It is also possible to administer the two main components with a time delay. Advantageously, therefore, the viruses or the cells producing viruses are administered firstly, preferably infecting the dividing cells of the organism and carrying within their genome at least one gene whose expression in the dividing cells will cause their destruction, followed by the substrates whose metabolization by the dividing cells will cause their destruction.

10 This elimination phase of the tumours mass is followed by an immunisation phase of the organism against this tumour by administration of cells, viruses or bacteria expressing the immunomodulators.

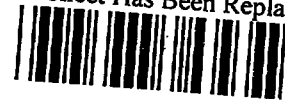
The composition according to the invention may be introduced by any means available to the man skilled in the art, particularly by syringe injection under medical imaging.

Advantageously this treatment is local but it may equally well be systemic.

20 The present invention is illustrated by the example below with reference to the appended single figure which represents the retroviral pMTK vector map.

EXAMPLE:

Treatment of mice with tumours using cells secreting
25 Interleukin-2 and producing the PMTK retrovirus.



C56 BL/6 mice with tumours were treated with a composition containing allogeneic cells producing Interleukin-2 and the PMTK retrovirus which carries the thymidine kinase gene of the HSV1 virus.

5 Injections were made in the vicinity of the tumours.

The pMTK vector (see figure) has the following characteristics:

LTR-5' - Mov3 as far as the BamH1 site (-350 from the start of transcription),

10 - Mov13 of the BamH1 site (-350) as far as the Kpn1 site (+ 30)

- Mov9 of the Kpn1 site (+ 30) as far as the Pst1 site (+ 560) (contains the packaging sequence

Ψ)

15 - The non-coding sequence at 3' of the Cla1 site (+ 7674) as far as U3 (+ 7817) is derived from Mov3.

LTR-3' - Mov3 as far as the BamH1 site positioned at 7910 (or-350),

- Mov13 as far as the end of US.

20 A Ψ CRIP packaging cell line is co-transfected with the pMTK plasmid (20μg) and the pWLNeO plasmid (1μg). Several clones were isolated at G418 selection.

The strength of the selected clone was 5.15^5 infections particles/ml as measured by the ability to
25 confer HAT resistance (Hypoxanthine, Aminopterin),



Thymidine) for L TK⁻ cells . The infection of L TK⁻ cells is performed with successive dilutions of the supernatant containing viral particles.

This Sheet Has Been Replaced



CLAIMS:

1. Composition for use in the treatment of tumours in human or animal organisms and to immunise these organisms against tumours, said composition comprising a synergistic association of:

- cells, viruses or bacteria transiently expressing in the organism at least one gene enabling them to produce in vivo one or more immunomodulators, and

- viruses, or cells producing viruses, said viruses preferably infecting dividing cells of the treated organism and carrying within their genome at least one gene whose expression in the dividing cells will cause their destruction.

2. Composition according to claim 1, characterized in that the cells produce in vivo one or more immunomodulators and moreover produce viruses carrying within their genome at least one gene whose expression in the dividing cells will cause their destruction.

3. Composition according to one of the claims 1 and 2, characterized in that the immunomodulators are Interleukin-2, Interleukin-4, Interleukin-7, TNF, Interferon-gamma, GM-CSF, alone or in combination.

4. Composition according to one of the claims 1 and 2, characterized in that the viruses carry a gene expressing thymidine kinase or cytosine deaminase.

This Sheet Has Been Replaced



14



5. Composition according to one of the claims 1 to 4, characterized in that the viruses are retroviruses, Adenoviruses, viruses associated with Adenovirus or Pox viruses.

5 6. Use of the composition according to one of the claims 1 to 5 for the manufacture of a medicinal product for the treatment of tumours and cancers and/or the immunization of the organism against these illnesses.

7. Use according to claim 6, characterized in that the
10 different components of the composition are present simultaneously or independently.

8. Use according to claim 6, characterized in that the
15 viruses, or cells producing viruses, preferably infecting the dividing cells of the organisms and carrying within their genome at least one gene whose expression in the
dividing cells will cause their destruction, are administered prior to or simultaneously with the administration of cells, viruses or bacteria producing the immunomodulators.

20 9. Use according to one of the claims 5 to 8, characterized in that the cells, viruses or bacteria producing the immunomodulators are replaced by any other means permitting the release of immunomodulators in the organism, such as a micropump.