Human Melanoma Gene Therapy: from Animal Studies to Clinical Trials

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1. Introduction

Gene therapy is one of the most rapidly developing fields of modern medicine closely related to the achievements in genetic engineering particularly to the development of the DNA (gene) transfer into eucariotic cells technology. During the last five years 120 clinical protocols of human gene therapy have been designed, which has involved about 600 patients. About half of the protocols are currently in the phase I or II clinical trials. In Europe, 6 out of 15 approved clinical protocols, including one in Poland, are currently curried out. Majority of protocols (60%) concern neoplastic diseases, 25% hereditary genetic disorders, 10% AIDS and remaining 5% rheumatic or vascular diseases (1).

Gene therapy may be defined as an alteration of the cell phenotype by insertion of "correct" or removal of "incorrect" genetic information or by modification of a normal cell by introduction of new information in order to control or treat the disease. Cells may be genetically modified *ex vivo* (cellular gene therapy) or *in vivo* (gene therapy).

A number of hereditary disorders are caused by a single gene defect which leads to the malformation of a particular metabolic pathway. In such cases, therapy would be based on the transfer of a functional copy of the gene into the defective cell. However, highest expectations regarding the development of the gene therapy are related to neoplastic diseases. Cancer

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therapy clinical protocols being in trial are based on 5 strategies: (i) genetic cellular cancer vaccines; (ii) introduction of major histocompatibility complex (MHC) antigens directly into tumor cells *in situ*; (iii) introduction of suicide genes into tumor cells *in situ* and activation of suicide mechanisms; (iv) introduction into tumor cells of suppressor genes and/or anti-oncogenes and blocking of oncogenes expression; (v) introduction of multidrug resistance genes (MDR) into bone marrow cells in order to protect them from high dose chemotherapy (1).

Gene therapy is based on vectors introducing DNA into target cells. Two types of vectors, viral and non-viral are currently applied in human trails. Non-viral vectors are cationic lyposomes. Viral vectors are based on retroviruses, adenoviruses, adenoassociated viruses and herpesviruses. In two clinical protocols "naked" DNA is injected into cells. In two/third of protocols retroviral vectors are employed. Retroviruses are RNA viruses. After internalization following binding to the surface receptors, viruses are decapsulated and the released RNA is transcribed to DNA (provirus), translocated into nucleus and integrated with the genome of the target cell. The viral genome consist of functional sequences (promotor, enhancer) and sequences coding structural proteins, and reverse transcriptase. In the retroviral vectors genes encoding viral proteins are replaced by the genes of interest. Usually, beside the therapeutical gene, antibiotic resistance gene is used. Removal of genes encoding structural proteins renders viruses replication defficient. However, those proteins might be provided *in trans* by the so called packaging cells. These cells produce empty capsules and pack into them RNA obtained from DNA of constructed vector transfected earlier into these cells. The obtained recombinant virus is able to infect (transduce) a wide range of target cells with high efficacy (up to 100% of cells) without destroing the cells and without further replication. The characteristic future of retroviral vectors is that they transduce only dividing cells and stably integrate introduced genes with the genome of the target cells. However, due to the variability of the gene expression level in different cell types, research is still carried out towards modificactions of the vectors such as application of strong egzogenious promoters or construction of dicistronic, or tricistronic vectors. Egzogenious promoters may be introduced both within transcriptional region or long terminal repeat (LTR) of the vector. In di- or tricistronic vectors two or three genes are joined by IRES sequences and driven by a common promoter which results in the production of two or three different proteins on the basis of one mRNA. In such cases, all transduced cells are producing all proteins of interest. However, there are some limitations of the system. There is a limit of a size of the genetic information carried by the vector. Another issue is the safety of the retroviral vectors. The probability of production of the so called 'helper virus' is very low. However, to eliminate this phenomenon, packaging cell lines are being modified. The widly applied line PA317 contains provirus in which the packaging sequence (psi) has been deleted, 5'LTR was removed and 3'LTR was replaced by the SV40 virus polyadenylation signal.

A larger number of cancer gene therapy clinical protocols deal with cancer cellular genetic vaccines. Historically, cellular tumor vaccines were used to treat patients for the past few decades. They consisted of either tumor cell lysates, irradiated autologous or allogeneic tumor cells or irradiated virus-infected cells (2). Immunization of patients with irradiated melanoma autologous cells resulted in clinical responses in 25% of patients (3) and stimulation of specific cytotoxic T-lymphocytes (CTL) (4). Polyvalent melanoma vaccine composed of 3 allogeneic cell lines induced IgM and IgG antibodies to melanoma associated antigens and in patients with stage IIIA and IV, melanoma increased survival 4-fold (5). The strategy of genetic cellular vaccines is to locally deliver, together with cancer cells, factors (most frequently cytokines) which will induce anti-cancer specific and non specific responses by enhancing presentation of tumor antigens or by providing costimulatory signals for the immune system. Cytokines might be provided by transfer of their genes into autologous tumor cells, allogeneic cell lines or fibroblasts which will be then mixed with autologous tumor cells (Tab. 1).

Autologous cellular genetic vaccines are prepared by the *ex vivo* transfer of cytokine genes into patient's own tumor cells cultured *in vitro*, which are then irradiated and subcutaneously injected back to the patient.

Allogeneic vaccines are based on cancer established cell lines which are transduced with genes encoding particular cytokine or other factors. In certain circumstances, such as problems with obtaining cancer tissue from the patient allogeneic vaccines, might be alternative to autologous vaccines. In general, preparation of autologous vaccines is difficult while allogeneic vaccines are believed to be less effective. Accordingly, efforts are undertaken to develop **mixed** vaccines. They comprise of autologous tumor cells, which are isolated from cancer tissue and frozen without genetic modification and cells producing cytokines. Autologous fibroblasts or allogeneic cell lines (as developed in our Department) modified to secrete cytokines may be employed (6).

Great antitumor potential in animal models has been demonstrated for the multifunctional cytokine IL-6. Initial studies (7) showed that IL-6 injected into mice prevented melanoma growth and metastasis formation. Combined therapy with IL-6 and inactivated 3LL lung carcinoma cells was even more effective in suppressing metastasis formation in mice (8). However, transfection of the IL-6 gene into tumor cells of various origins has had a more pronounced effect in tumor rejection and metastasis prevention than systemic IL-6 administration (9). A number of ongoing human clinical trials have shown that systemic IL-6 administration has no significant anti-melanoma potential (10). Tumor inhibitory actions of IL-6 appear to be T-cell dependent; $CD4^+$ and $CD8^+$ cells were required for the regression of established pulmonary metastases of weakly immunogenic fibrosarcoma in mice following systemic administration of IL-6 (9). Moreover, lung metastatic potential of a weakly immunogenic clone of the Lewis lung carcinoma was significantly decreased following vaccination with tumor cells transfected with the IL-6 cDNA (9). Costimulatory effects of a number of cytokines in-

TABLE 1
PROTOCOLS OF CANCER GENE IMMUNOTHERAPY

Cells	Advanced cancer	Melanoma	Renal ca	Neuroblastoma Glioblastoma	Lung ca	Breast ca	Prostate ca	Colon ca
TIL[a]	IL-2 TNF	IL-2+IL-4 IL-2+INFγ[b]						
CIK[c]	IL-7	IL-4						
Autologous	IL-2 TNF	IL-2 IL-7 INFγ GM-CSF	GM-CSF	IL-2 IL-2[d] INFγ	IL-2	IL-2	GM-CSF	
Allogeneic		IL-2(3)[e] IL-4 B7	IL-2				IL-2+INFγ	
Allogeneic + autologous		IL-6+sIL-6R						
Fibroblasts + autologous	IL-4							IL-2
Fibroblasts to tumor		IL-12						
Directly to tumor	IL-2	B7(2)[e]						B7

[a]TIL — tumor infiltrating lymphocytes; [b]melanoma and renal carcinoma; [c]CIK — cytokine induced kiler; [d]glioblastoma; [e]number of protocols.

cluding IL-6 in antitumor activities were mainly linked to cytotoxic T lymphocytes (9,11). However, IL-6 may also activate the NK cell response (12). More detailed *in vitro* analysis demonstrated that in combination with IL-1, IL-6 costimulated resting CD4+ T cell populations in the absence of activation of the CD28 molecule (13). Costimulation of CD4+ T cells *in vivo* by IL-6-transfected melanoma cells and development of anti-melanoma immunity was shown in our studies which employed ß2-microglobulin deficient mice (see below) which lacked CD8+, but possessed CD4+ and NK cells (14). However, in many circumstances IL-6 is not an essential co-stimulant for T cells *in vivo*.

IL-6 exerts its activity through a membrane bound receptor complex consisting of IL-6 low affinity binding protein (gp80, IL-6Rα chain, CD126) and gp130 (CD13). IL-6 first binds to IL-6Rα and then the complex attracts two gp130 molecules which covalently link via disulfide bonds (15) which finally leads to signal transduction. Moreover, soluble forms of both α and β subunits were found. SIL-6Rα was able to bind IL-6 and trigger homodimerization of membrane gp130 which led to signal transduction. A complex of a sIL-6R and IL-6 exhibits activities distinct from those of IL-6 alone *in vitro*, i.e. it may elicit a specific signal on cells which express gp130 only (16,17). Moreover, sIL-6R enhances IL-6 activity *in vitro* (18). In turn, soluble gp130 was able to bind to IL-6/sIL-6Rα complex and inhibit its activity (19). Very recently, studies of complex formation of IL-6 with the soluble α and β chains of the receptor have indicated that an active receptor complex may consist of two molecules of each IL-6, α- and β-chains forming a hexameric structure (20). Both soluble forms of gp80 and gp130 have been found in the circulation but their biological role *in vivo* still remains obscure (19,21).

2. Background

The incidence of malignant melanoma is increasing steadily in Poland as well as in other countries. The survival of patients in whom primary tumors have been surgically excised at an early stage of the disease (histological grade Clark I or II) is relatively satisfactory. However, prognosis of the metastatic form of the disease is very poor with a mean survival time of patients with clinical stage IIIA or IV of disease being 8 months. Early primary lesions of melanoma are frequently ignored by patients in Poland and the majority of surgically removed primary tumors are of advanced histological grade (Clark IV or V), very often accompanied by metastases in proximal lymph nodes. While early stage melanoma might be successfully treated by surgery the metastatic form of the disease is resistant to conventional therapy which includes hormonal therapy, chemotherapy or radiation. Chemotherapy, while transiently effective in a small number of patients, does not increase the survival time of these patients (reviewed in 2).

3. Experimental

3.1. Animal studies

B-78-H1 murine melanoma cells (H-2K^b negative) were stably transfected with cDNAs coding for human IL-6, murine sIL-6R or the empty vector pCDM8. They constitutively secreted about 100 ng/ml/10^6 cells/24 h of IL-6 or sIL-6R. C57BL/6 x C3H (H-2 bxk) mice, severe combined immunodeficient (SCID) CB17 (H-2b) mice or β2-microglobulin (β2-m) knockout mice deficient in the maturation of class I MHC-restricted CD8$^+$T cells (homozygous (-/-) x C57BL/6 F3) were injected subcutaneously (s.c.) with 5 x 10^5 viable control (mock transfected) or IL-6 and sIL-6R transfected B-78 cells. C57BL/6 x C3H severe combined immunodeficient (SCID) mice were also injected intravenously (i.v.) into the tail vein with 5 x 10^5 control or transfected cells (18).

In another set of experiments C57BL/6 x C3H mice were initially injected s.c. with transfected B-78 or control cells (left flank) and, after 2 weeks, challenged with parental B-78 cells (right flank). Mice were maintained for 4 months and tumor formation and survival were monitored. The same strain of mice was initially injected i.v. with B-78 parental cells and after 10 days subsequently challenged s.c. with B-78 or B-78-transfected cells. Animals were sacrificed 4-5 weeks following the first injection. The presence of lung metastases was assessed using light microscopy.

Insertion of IL-6, sIL-6R, and a combination of IL-6 and sIL-6R (1:1 mixture) into B-78 cells caused a significant reduction in the tumor diameter. The combination of IL-6 and sIL-6R was the most effective treatment regimen. The kinetics of tumor growth and survival of C57BL/6 x C3H mice demonstrated that all animals injected s.c. with control cells developed tumors by 4 weeks following injections. At this time point, only half of the mice injected with B-78 transfected with IL-6 or sIL-6R had tumors. Until the 7th week none of the animals injected with a mixture of B-78-IL-6 and B-78-sIL-6R cells (1:1) showed a palpable tumor. In Figure 1. tumor diameters on the 8th week following injection are shown. Survival analysis demonstrated increased survival time of mice injected with B-78-IL-6 and a mixture of B-78-IL-6 and B-78-sIL-6R cells when compared with mice injected with B-78 or B-78-sIL-6R cells alone.

I.v. injections of control cells into C57BL/6 x C3H mice after 4-5 weeks resulted in massive metastases growth in lungs of all animals. In mice injected with B-78-IL-6 and B-78-sIL-6R cells, significantly fewer lung metastases were found. Animals injected with a mixture of cells transfected with IL-6 and sIL-6R showed almost no metastasis formation. More animals injected with transfected cells in comparison to those injected with control cells survived 5 weeks.

All mice immunized with control and sIL-6R-transfected cells challenged after 2 weeks with parental B-78 cells developed tumors after 8 weeks. However, 80% of the animals immunized with B-78-IL-6 cells and only 20% of

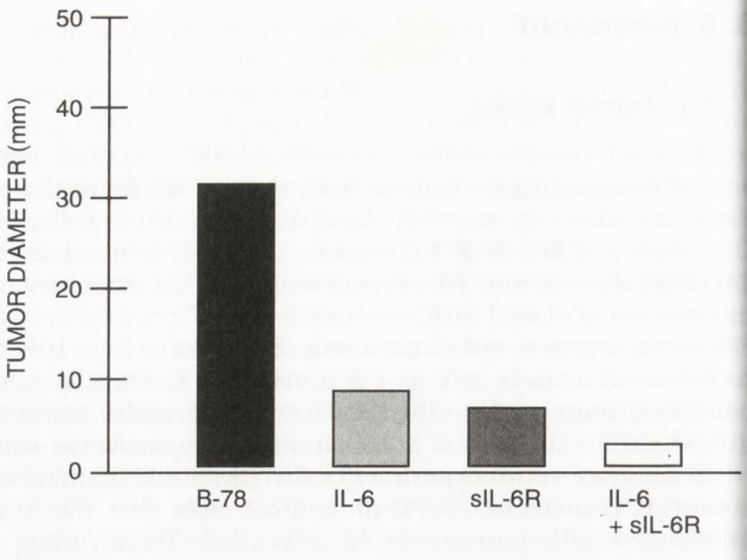

Fig. 1. Tumor formation in mice injected subcutanously with control and transfected B-78 cells.

those immunized with a mixture of IL-6- and sIL-6R-transfected B-78 cells had tumors. This status did not change during 16 weeks of observation. Survival analysis demonstrated significant differences between the groups studied. Ninety percent of mice immunized with a mixture of IL-6 and sIL-6R B-78 cells in comparison to only 10% of control animals survived 16 weeks.

Subsequent s.c. challenge of transfected melanoma cells into animals initially injected i.v. with B-78 control cells resulted in a reduction of lung metastasis when compared to subsequent s.c. challenge with untransfected cells.

To evaluate the applicability of the studied factors for the construction of genetic cellular vaccines, animals were initially injected i.v. with B-78 melanoma cells. Ten days later they were challenged by injection (s.c.) with the transfected cells. Such procedure resulted in an increase in the number of animals surviving 5 weeks and a reduction in the number of lung metastases.

SCID mice injected with control cells developed heavy lung metastases and more than 50% of animals died within 5 weeks. Similarly, B-78-IL-6 cells formed lung metastases. Transfection of sIL-6R into B-78 cells resulted in significantly reduced metastasis formation by these cells and increased survival of the animals. The combination of B-78-IL-6 with B-78-sIL-6R did not exceed the effect of B-78-sIL-6R alone.

All β2-m knockout mice injected s.c. with control cells developed tumors and died after two weeks following injection. Only 50% of the animals injected with B-78-IL-6 cells had tumors which were significantly smaller. These mice died 4 weeks after cell injection. The remaining 50% of the animals which did not develop tumors were re-challenged s.c. with parental cells. None developed tumors. B-78 transfected with sIL-6R were not tested in this model (14).

Conclusions drawn from animal studies: (i) sIL-6R gene introduced into melanoma cells inhibits their growth, and their ability to metastasize, and stimulates potent, specific, and long lasting anti-melanoma immunity; (ii) the activity of the sIL-6R is linked to the presence of IL-6 either secreted by B-78 cells or produced by the host in response to injected tumor cells (22), this assumption is supported by the fact that in the system used the most pronounced biological effect of the sIL-6R was observed when sIL-6R producing cells were combined with those producing IL-6; (iii) the complex of IL-6/sIL-6R significantly affected growth kinetics compared to the effects displayed by IL-6 or sIL-6R alone. However, survival time analysis demonstrated no significant difference between the animals injected with B-78-IL-6 cells and those injected with the combination of IL-6 and sIL-6R secreting cells; (iv) preimmunization experiments have clearly showed a significantly higher potential of the IL-6/sIL-6R complex over IL-6 alone in stimulating specific and long lasting anti-melanoma immunity; (v) transfection of sIL-6R into B-78 cells resulted in increased inhibition of metastasis formation in comparison to transfection of IL-6 into B-78 cells in C57BL/6 x C3H mice. A marked inhibitory effect was observed when cells transfected with IL-6 and with sIL-6R cDNA were injected together.

The observed difference in the biological activity of IL-6 versus the IL-6/sIL-6R complexes may be explained by the (i) enhancement of IL-6 activity by sIL-6R on cells possessing gp80 and gp130 receptor subunits and (ii) involvement of cells which only express gp130 and are therefore nonresponsive to IL-6 alone but respond to the complex of IL-6/sIL-6R. Since combined secretion of IL-6 and sIL-6R inhibited melanoma growth in the early phase of the dissease and IL-6/sIL-6R complexes were protective in metastasis formation in SCID mice, it is likely that one of the target cells of IL-6/sIL-6R is the NK cell. This is supported by recent reports demonstrating that for protection against tumors induced by the injection of plasmacytoma cells secreting a number of cytokines, T cells are not required during the first 6-7 weeks (23). Another target cell is the T lymphocyte since IL-6/sIL-6R complexes have greater potential to stimulate specific antitumor immunity than IL-6 alone. Finally, it is likely that IL-6/sIL-6R activates antigen presenting cells such as dendritic cells or macrophages.

3.2. Clinical Protocol

Since melanoma is considered to be one of the most immunogenic malignant tumors in man it has become the primary target for gene immunotherapy approaches. The majority of the approved clinical protocols of melanoma gene therapy are based on the transduction of a variety of cytokine genes into autologous tumor cells. However, deriving a sufficient quantity of autologous cells to be transduced in a relatively short time presents a difficulty. Moreover, these cells usually divide rather slowly, thereby reducing the efficiency of transduction using retroviral vectors. Subsequent selection of positive cells reduces total cell number. Finally, in about 30%

of patients establishment of tumor cell lines is not possible at all. Moreover, several melanomas lack or have defective expression of MHC class I molecules which are necessary for the presentation of melanoma antigens to autologous T lymphocytes. In view of the facts presented above, an allogeneic melanoma cell-based approach has been devised. Approximately 70% of the European population is HLA-A1 and/or HLA-A2 positive. Accordingly, in the majority of patients, application of an allogenic approach with HLA-A1 or HLA-A2 restriction should be feasible.

3.2.1. Objectives

To actively immunize HLA-A1 and/or HLA-A2 positive patients with irradiated autologous tumor cells admixed with HLA-A1,A2 positive allogeneic melanoma cells that were genetically modified by transduction to secrete interleukin 6 (IL-6) and soluble IL-6 receptor (sIL-6R), it is necessary:

1. To evaluate local and systemic toxicity of multiple subcutaneous (s.c.) injections of the mixture of autologous and IL-6/sIL-6R — producing allogeneic tumor cells.
2. To evaluate the induction or augmentation of tumor-specific and non-specific immune responses.
3. To assess potential anti-tumor effects of the treatment.

3.2.2. Preparation of autologous cells secreting IL-6 and sIL-6R

Double copy dicistronic retroviral vector based on MSCV (murine steem cell virus) was constructed (24). Cassette containing CMV promoter with enhancer and cDNA coding IL-6 or sIL-6R linked with Neo resistance gene using IRES sequence was inserted into U3 region of 3'LTR. Metastatic human melanoma cell line which was HLA-A1,2 and MAGE 1 and 3 positive, was transduced with IL-6 or sIL-6R carring vectors. After selection two cell populations, one secreting approximately 100 ng/ml/10^6 IL-6 the other secreting similar quantity of sIL-6R were obtained. Both sublines were maintained and propagated separately. Prior to irradiation they were mixed 1:1 to the total cell number of 2.5 x 10^7.

3.2.3. Vaccine preparation

Metastatic lesions were surgically removed and tissue minced into 2-3 mm pieces. Cells were isolated by enzymatic digestion with collagenase and separated by density gradient centrifugation in Percol. The melanoma cell-rich fraction was collected and cells frozen in aliquots containing 2.5 x 10^7 cells. Prior to injection autologous cells were admixed with the same amount of allogeneic cells secreting IL-6 and sIL-6R and irradiated with 100 Gy. Before irradiation, an aliquot of the mixture was examined for bacterial, fungal and mycoplasma contamination.

3.2.4. Treatment Schedule

Patients were injected in two week intervals at day 0, 14, 28, 32, and then once a month for 3 consecutive months. Subsequent injections were administered at the discretion of the investigator depending on the clinical response to the treatment. One dose contained total of 5×10^7 cells prepared as described above. Cells were injected at 4 different sites in the area of draining of non affected lymph nodes.

3.2.5. Evaluation of the Response to the Treatment

Clinical response to the treatment was evaluated on the basis of physical examination, X-ray, US or CT and NMR-scans. The following criteria were used:

A. Complete Response (CR) — complete remission of the disease for at least 1 month.

B. Partial Response (PR) — at least 50% of remission of total tumor burden.

C. Stable Disease (SD) — less than 25% of remission for at least 3 months.

D. Progressive Disease (PD) — progression of existing tumor mass by at least 50% and/or appearance of new lesions.

The time of the response was measured from the moment when CR and PR were first recorded to the point of the PD. Survival was measured from the moment when the first metastasis was first detected.

3.3. Preliminary results

Since 6th of January 1995 five stage IV melanoma patients were enroled into phase I (toxicity) clinical trial. They all were HLA-A1 or HLA-A2 positive. In one patient (E.U.) melanoma cells were Mage 1 and 2 positive, in one patient (T.W.) Mage 1 positive, and in one patient (A.L.) MAGE 2 and 3 positive. In two patients melanoma cells were MAGE negative. In two patients E.P. and T.W. multiple skin metastases after 3rd injection changed color and temperature. Serial biopses taken from these foci demonstrated heavy infiltrates of CD8+ and CD4+ lymphocytes, and macrophages which kept increasing following next injections. However, only in patient T.W. some metastases regresed. In patient A.L. who was enroled into the studies in the terminal stage of melanoma after the 4th injection *vitiligo* at the face was observed. Patient E.U. developed mediastinal lymphnode metastases (7-11 cm/diameter) and left ovary metastasis about 20 cm/diameter. Three months after surgical removal of ovarial metastasis patient was enroled into the study. Mediastinal metastases completely regressed and the patient is tumor free until now. Charateristics of the patients are presented in Table 2. Immunological parameters evaluated in the patients enroled into the study are presented in the same volume (25).

TABLE 2
PHASE I (TOXICITY) STUDY

Patient (age)	Stage of Disease	Sites of Metastases	Time from the 1st metastatic focus (months)	Time of Treatment (months)	Toxicity	Clinical Response
E.P. (32)	IV	S, LN, L, Li	36	5	no	PD
T.W. (50)	IV (terminal)	S, LN	8	4.5	no	PR
A.L. (45)	IV (terminal)	S, LN, L, B	6	1.5	no (fever)	PD
E.U. (23)	IV	S, LN, O	60	9-pending	no	CR
J.W. (25)	IV (terminal)	S, LN, L	18	3	no	PD

S — skin, LN — lymphnodes, L — lungs, Li — liver, O — ovary, B — brain.

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Summary

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A number of hereditary disorders are caused by a single gene defect what leads to the malformation of the particular metabolic pathway. In such cases, therapy would be based on the transfer of functional copy of the gene into defective cell. However, highest expectations regarding the development of the gene therapy are related to the neoplastic diseases. Cancer therapy clinical protocols being in trial are based on 5 strategies: (i) genetic cellular cancer vaccines; (ii) introduction of major histocompability complex (MHC) antigens directly into tumor cells *in situ*; (iii) introduction of suicide genes into tumor cells *in situ* and activation of suicide mechanisms; (iv) introduction into tumor cells of suppressor genes and/or antioncogenes and blocking of oncogenes expression; (v) introduction of multidrug resistance genes (MDR) into bone marrow cells in order to protect them from high dose chemotherapy.

A large number of cancer gene therapy clinical protocols deal with cancer cellular genetic vaccines. Strategy of these vaccines is to locally deliver, together with cancer cells, factors (most frequently cytokines) which will induce anti-cancer specific and non specific responses by enhancing presentation of tumor antigens or by providing costimulatory signals for immune system. Cytokines might be provided by transfer of their genes into autologous tumor cells, allogeneic cell lines or fibroblasts which will be then mixed with autologous tumor cells.

Interleukin 6 (IL-6) displays its activity through a membrane specific receptor composed of two subunits α (gp80, CD126) and β (gp130, CD130). Soluble form of gp80 (sIL-6Rα) acts agonistically with IL-6. Transfer of IL-6 and sIL-6Rα genes into murine melanoma cells results in the inhibition of tumor growth and metastases formation. Immunization of mice with IL-6/sIL-6Rα transduced melanoma cells induced long lasting, specific anti-melanoma immunity. Based on the preclinical studies clinical protocol for immuno-gene therapy of human melanoma was designed in our Department. In January 1995, phase I clinical trial was initiated. Until now 5 patients with IV clinical degree of melanoma received genetic vaccine. 2.5×10^7 autologous cells were mixed with the same amount of allogeneic cells modified to secrete IL-6 and sIL-6R and injected to patients according to the following schedule: 4 injections in two weeks intervals, 3 injections in one month intervals and 3 injections in two months intervals. During therapy no toxic effects were observed. Induction of specific and non-specific anti-melanoma response was observed. Currently the trial enters phase II. Optimalization of doses and immunization schedule as well as verification of patients eligibility will be carried out. Moreover, clinical effects of applied therapy will be monitored.

Key words:
gene therapy, cellular genetic vaccines, cytokines, melanoma, interleukin 6, soluble interleukin 6 receptor.

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