

study protocol

Treatment of skin tumors with intratumoral interleukin 12 gene electrotransfer in the head and neck region: a first-in-human clinical trial protocol

Ales Groselj^{1,2}, Masa Bosnjak^{3,4}, Tanja Jesenko^{2,3}, Maja Cemazar^{3,5}, Bostjan Markelc^{3,6}, Primož Strojani^{2,7}, Gregor Sersa^{3,6}

¹ Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana, Ljubljana, Slovenia

² Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

³ Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia

⁴ Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

⁵ Faculty of Health Sciences, University of Primorska, Izola, Slovenia

⁶ Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia

⁷ Department of Radiation Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia

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Ales Groselj and Masa Bosnjak have contributed equally to this work and share first authorship.

Correspondence to: Prof. Gregor Serša, Ph.D., Institute of Oncology Ljubljana, Zaloška 2, SI-1000 Ljubljana, Slovenia. E-mail: gsertsa@onko-i.si

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Background. Immune therapies are currently under intensive investigation providing in many cases excellent responses in different tumors. Other possible approach for immunotherapy is a targeted intratumoral delivery of interleukin 12 (IL-12), a cytokine with anti-tumor effectiveness. Due to its immunomodulatory action, it can be used as an immunostimulating component to in situ vaccinating effect of local ablative therapies. We have developed a pHL12 plasmid devoid of antibiotic resistance marker with a transgene for human IL-12 p70 protein. The plasmid can be delivered intratumorally by gene electrotransfer (GET).

Patients and methods. Here we present a first-in-human clinical trial protocol for pHL12 GET (ISRCTN15479959, ClinicalTrials NCT05077033). The study is aimed at evaluating the safety and tolerability of pHL12 GET in treatment of basal cell carcinomas in patients with operable tumors in the head and neck region. The study is designed as an exploratory, dose escalating study with the aim to determine the safety and tolerability of the treatment and to identify the dose of plasmid pHL12 that is safe and elicits its biological activity.

Conclusions. The results of this trial protocol will therefore provide the basis for the use of pHL12 GET as an adjuvant treatment to local ablative therapies, to potentially increase their local and elicit a systemic response.

Key words: gene therapy; interleukin 12; gene electrotransfer; basal cell carcinoma; head and neck region

Introduction

Immune therapies are currently under intensive investigation. Immune checkpoint inhibitors became standard of care for variety of tumor types, providing in many cases excellent responses in patients

with advanced or progressive disease, unsuitable for treatment with local therapies. However, there are still patients that are non-responders and the reasons for that are still not well understood.¹

The other category of immune stimulators are cytokines. These have been extensively investigat-

ed, predominantly in combined treatment schemes. The IL-12 is a soluble cytokine with anti-tumor effectiveness. It stimulates adoptive and natural immunity in the organism, predominantly through production of interferon gamma (IFN- γ).² Besides immunostimulatory effectiveness it also has anti-angiogenic mode of action.² Treatment with recombinant IL-12 has confirmed its anti-tumor effectiveness, but due to the high drug concentrations required to induce effect also severe toxicity were observed, which eventually lead to abrogation of IL-12 use.³

Gene therapy provides a new technical approach for more localized delivery of the therapeutic proteins to tissues, therefore reducing their eventual systemic toxicity. In most of the clinical trials, genes are delivered by viral vectors; however, non-viral gene delivery systems like gene electrotransfer (GET) also provide a safe approach for naked plasmid DNA delivery to tumors.^{4,5} GET is based on electroporation that uses electric pulses to destabilize the cell membrane for delivery of large and non-permeant molecules into the cells.⁶ The transport of plasmids through the cell membrane and into the nucleus for transcription is not well understood.⁷ However, there are several clinical studies providing evidence of feasibility, safety, and effectiveness of this non-viral gene delivery.^{4,5,8,9}

Studies on murine tumor models have shown that GET with plasmid DNA encoding interleukin 12 (IL-12 GET) is especially successful in the treatment of skin tumors and their metastases.¹⁰ Furthermore, the safety and efficacy data of such treatment on skin melanoma metastases were already published.⁴ In a human clinical study with IL-12 GET of melanoma metastases, local and also systemic effectiveness on the distant non-treated tumors were demonstrated. The study included 24 patients with skin metastatic melanoma.⁴ The plasmid encoding human IL-12 under the control of the CMV promoter and with resistance to kanamycin, as the selection gene, was used. Gene therapy was performed three times on each tumor, resulting in promoted local clinical response of treated tumors and in systemic anti-tumor effect on distant non-treated nodules in 53% of patients. This treatment approach is currently being investigated in the treatment of melanoma, Merkel cell carcinoma, breast cancer^{8,9,11,12}, and also in combination with immune checkpoint inhibitors.^{13,14}

However, despite these encouraging results, IL-12 plasmid used in the described studies contain antibiotic resistance gene serving as a selection

marker for the production of plasmid DNA. As the presence of antibiotic-resistance genes raises safety concerns, European Union regulatory requirements endorse the use of plasmids without the selective genes. Therefore, we developed the pHIL12 plasmid and its clinical grade production process in our previous studies.^{15,16} In the proposed first-in-human study (EudraCT: 2021-000852-21, ISRCTN15479959, ClinicalTrials NCT05077033) we intend to study the safety and tolerability of pHIL12 GET in treatment of basal cell carcinomas in patients with operable tumors in the head and neck region. The study is designed as an exploratory, dose escalating study with the aim to determine the safety and tolerability of the treatment and to identify the dose of plasmid pHIL12 that is safe and elicits its biological activity.

Trial rationale

Basal cell carcinoma accounts for 80% of all non-melanoma skin cancers and is the most common malignant tumor that occurs on the skin. It grows slowly and rarely metastasizes. However, basal cell carcinoma comprises heterogeneous histologic variants, from highly biologically benevolent, well-limited and superficially growing tumors to aggressive, infiltratively growing or deeply invasive lesions. Due to complex anatomical conditions in some parts of the head (nose, orbital area, ears) combined with a possibly more aggressive form of growth, an inadequate first treatment may present a serious therapeutic problem. The basic therapeutic options in basal cell carcinoma are surgery, radiotherapy and electrochemotherapy, that result in comparable local control when early lesions are treated.¹⁷ Since the baseline tumor assessment could be inadequate and the disease underestimated, relapses at the treatment site are not uncommon. Repeated treatment, whether surgical or radiotherapeutic, is associated with reduced efficacy and/or functional or cosmetic impairment in the treated area. Therefore, from clinical perspective, searching for new therapeutic alternatives is of high importance. Recently, immunotherapy became important for the treatment of locally advanced or metastatic basal cell carcinoma, with first approved immune checkpoint inhibitor cemiplimab, which has set the stage for investigation of other immunotherapies.¹⁸

Basal cell carcinoma has the highest mutational burden compared to other skin cancers resulting in the activation of the local immune response that

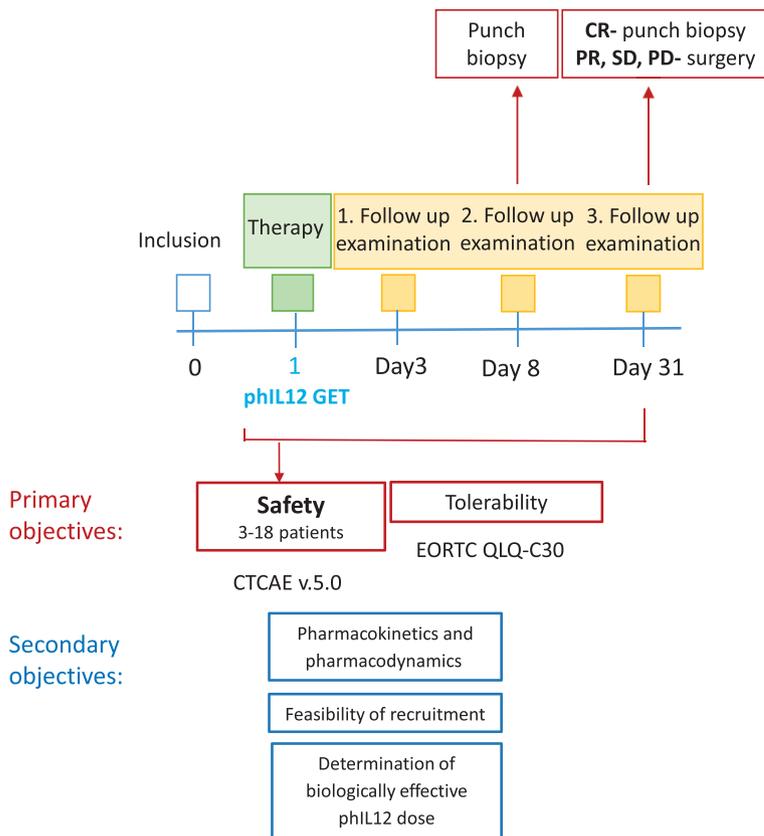


FIGURE 1. Clinical trial design.

CTCAE v.5 = Common Terminology Criteria for Adverse Events version 5.0; CR = complete response; EORTC QLQ-C30 = European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire C30; PR = partial response; PD = progressive disease; SD = stable disease

prevents rapid tumor growth and metastasis.¹⁹ Indeed, clinical studies confirmed that electrochemotherapy is more effective in basal cell carcinoma than in other histological tumor types.^{20,21} This can be explained by a higher mutational load and an increased presence of tumor antigens or neoantigens that are triggered after electrochemotherapy and are required for effective stimulation of immune system and elimination of tumor cells.²²

In accordance with these findings we have designed the proposed clinical study. It is based on GET of plasmid DNA encoding IL 12 into the tumor, aiming to subsequently stimulate local immune response that would result in tumor eradication. If this therapeutic approach with IL-12 will prove to be safe and effective, it will be the first “proof of principle” of its kind in clinic and a valuable addition to the existing therapeutic armamentarium. However, in the case that our study will confirm the safety of the proposed therapy, but

will not yield expected therapeutic response, the tumors will be treated with standard treatment, i.e. surgery. The prolonged interval to surgery should not affect prognosis of treatment outcome, due to slow course of basal cell carcinoma growth.²³

Trial design

This is a clinical, interventional, open label, single arm, Phase I trial for intratumoral pHIL12 GET. It is intended to treat basal cell carcinomas in patients with operable tumors in the head and neck region. The aim of the study is to evaluate the safety and tolerability of pHIL12 GET. The study is designed as an exploratory, dose escalating study with the aim to determine the dose that produces IL-12 expression in the tumors with best biological activity, infiltration of the immune cells and no toxicity. Study hypothesis: Intratumoral pHIL12 GET is safe and tolerable in treatment of skin tumors (Figure 1).

The aim will be achieved with i) controlled application of the treatment, ii) evaluation of trial and obtained clinical results, iii) presentation of the results by providing written and audio-visual material, participation at and organization of expert meetings and scientific meetings.

In the study 3–6 patients per IL-12 dose level will be included; 3 doses, for a total estimated number of 9 patients (depending on the course of the study, from 3 and up to 18 patients will be included). The enrollment of the patients will be staggered: the waiting period will be 30 days after the treatment of previous patient, based on expected duration of acute and subacute toxicity. Consecutive cohorts of 3 to 6 patients will be treated with increasing doses of pHIL12 at three dose levels (0.5 mg/ml, 1 mg/ml and 2 mg/ml) according to an adapted 3 + 3 design as described below. There will be no intra-patient dose escalation. The clinical study will be conducted in accordance with this protocol and GCP guidelines and in accordance with the regulations.

The study is conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Institute of Oncology Ljubljana (protocol code ERIDEK-0086/2020, date of approval 25 November 2020). The study was also approved by the National Ethics Committee of the Republic of Slovenia (0120-524/2020-12) and the Agency for Medicinal Products and Medical Devices of the Republic of Slovenia. The study was registered in the ISRCTN (ISRCTN15479959) and Clinical Trials (NCT05077033) database.

TABLE 1. Primary objectives

Primary objective	Definition of objectives	Timepoint of objectives evaluation
Assessment of the safety of intratumoral pHIL12 GET	Assessment of adverse events in accordance with the CTCAE v5 criteria	From the beginning of therapy until the follow-up examination on day 30 after the treatment (day 1, 3, 8 and 31)
Assessment of the tolerability of intratumoral pHIL12 GET	Assessment of patient reported outcome by the quality of life questionnaire EORTC QLQ-C30	A follow-up examination on day 0, 8 and 31

CTCAE = Common Terminology Criteria for Adverse Events; GET = gene electrotransfer

TABLE 2. Secondary objectives

Secondary objective	Definition of objectives	Timepoint of objectives evaluation
Pharmacokinetics and biodistribution.	Determination of serum levels of IL-12 cytokine.	A follow-up examination according to clinical trial protocol (day 0, 3, 8 and 31).
Pharmacodynamics	Determination of tumor IL-12 and IFN- γ levels in tumor biopsies. Determination of plasmid DNA in tumor biopsies.	A follow-up examination according to clinical trial protocol (day 8 and 31).
Feasibility of recruitment	Evaluation of the appropriateness and execution of the treatment and follow up procedures.	During recruitment, execution of the treatment and follow up.
Determination of recommended dose for confirmatory studies	Measurement of pharmacodynamics data and selection of the pHIL12 dose that produces IL-12 expression in the tumors with best biological activity, infiltration of the immune cells and no toxicity.	Based on all measurements during follow up.

Objectives

Primary and secondary objective of the study are presented in Table 1 and Table 2, respectively.

Inclusion and exclusion criteria

In the study, only patients with confirmed basal cell skin carcinoma of the head and neck will be included. Their inclusion eligibility will be assessed in accordance with the inclusion and exclusion criteria (Table 3).

Trial procedures

Inclusion of patients

Patients with basal cell skin carcinoma of the head and neck, discussed at the multidisciplinary oncology advisory team meeting where all patients with confirm malignancies in the head and neck are presented and discussed, will be reviewed and informed of their eligibility to participate in the SmartGeneH&N clinical study. They will be presented with all information (in written and oral form) regarding the study and other possible treatment options. Prior to inclusion, they will sign an informed consent form. The patients included in

the study will be the one that meet all the inclusion criteria and will not have any exclusion criteria (Table 3).

The enrollment of the patients will be staggered. The waiting period between each individual will be 30 days after completion of therapy, based on expected duration of acute and subacute toxicity.

Consecutive cohorts of 3 to 6 patients will be treated with increasing doses of pHIL12 at three dose levels (0.5 mg/mL, 1 mg/mL and 2 mg/mL) according to the adapted 3 + 3 design (see below). There will be no intra-patient dose escalation. Patients will be assigned to a treatment cohort and will receive pHIL12 at a single dose-level.

If no dose limiting toxicity (DLTs) are observed during the therapy, until day 30 after the treatment (day 31), in the first 3 patients treated at a dose level, 3 additional patients will be enrolled and treated at the next higher dose level. Doses will be escalated until ≥ 2 of 3 patients (67–100%) in a dose cohort have at least 1 DLT. If exactly 1 of the first 3 patients treated at a dose level experiences at least one DLT, 3 additional patients will be enrolled and treated at that dose level. If none of the additional 3 patients (i.e., 1 of 6 [16.7%] total patients in this dose cohort) experiences at least 1 DLT, dose escalation may proceed. If any patient of the additional

TABLE 3. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Histologically or cytologically confirmed, previously untreated cutaneous basal cell carcinoma located in the head and neck region	Other malignancy at the time of inclusion
Solitary tumors, with largest diameter up to 3 cm, in the region where curative (R0) surgery is feasible	Lesions not suitable for treatment with GET (invasion into the bone, infiltration of large vessels)
Age 18-years or older	A life-threatening infection and/or severe heart failure and/or liver failure and/or other life-threatening systemic diseases
Life expectancy > 3 months	Significantly reduced lung function, which requires the determination of DLCO. Patients should not be treated if DLCO is abnormal
Physical performance in accordance with the Karnofsky scale ≥ 70 or < 2 in accordance with World Health Organization (WHO) scale	Treatment with immunosuppressive drugs, steroids and other drugs that would affect poor wound healing
The patient must be capable of understanding the treatment procedure and possible adverse events, which may arise during treatment	Age under 18-years
The patient must be capable of signing the informed consent to participate in the clinical study (voluntary and conscientious consent after education)	Major disruptions in the coagulation system (who does not respond to the standard therapy – replacement of vitamin K or freshly frozen plasma)
Prior to inclusion in the trial, the patient must be presented at a multidisciplinary advisory team meeting	A chronic decline in the kidney function (creatinine > 150 $\mu\text{mol/L}$)
	Epilepsy
	Pregnancy and breast-feeding
	The patient's incapability of comprehending the purpose or course of the trial, or not agreeing to be included in the trial
	Patients unwilling or unable to comply with the protocol requirements and scheduled visits

DLCO = Diffusing Capacity of the Lungs for carbon monoxide; GET = gene electrotransfer

3 patients (i.e., ≥ 2 of 6 [33.3%] total patients in this cohort) experiences at least 1 DLT, dose escalation will be stopped.

A DLT is defined as any grade 4 clinical or biological event related to the study treatment and occurring during the first 30 days after the treatment with pHIL12 GET.

Randomization

This is a one arm, open label clinical study without randomization.

Treatment

The application of the general or local anesthesia or sedation will be selected by the anesthesiologist. The pHIL12 will be injected intratumorally, and 5 minutes thereafter the electric pulses will be applied to the tumor for the transfection of tumor cells. Drug dosage and regime was determined based on non-clinical data. A single dose of pHIL12 will be administered by intratumoral injection. In the study we will use three doses of pHIL12: 0.5 mg/ml, 1 mg/ml, 2 mg/ml, which were determined based on non-clinical data. The lowest dose, 0.5 mg/ml, is the dose that, according to the guidelines of the

European Medicines Agency (EMA), elicits a pharmacological effect in the mouse model in preclinical testing. The next two doses are also determined in accordance with the guidelines with 2 mg/ml being the maximum feasible dose (MFD). Preclinical testing of all three doses has shown satisfactory results in mice that received a single pmIL12 GET therapy (mouse orthologue of pHIL12).¹⁵ An electrical pulse generator CLINIPORATOR™ (IGEA, s.p.A.) holding authorization for the use in the clinical environment with designation CE, will be used. It generates electric pulses appropriate for pHIL12 GET. The electrodes of the same manufacturer with parallel row needle array will be used. The application of electric pulses will be performed as described in the updated Standard Operating Procedures where a detailed information on electrodes and their usage in different clinical aspects is defined.²⁴ At the end of the treatment, we will take care of the lesions with standard wound-dressing techniques and move the patient into the post-anesthetic care for further monitoring and pain management. When patient will recover from anesthesia, treatment toxicity, possible side effects (Common Terminology Criteria for Adverse Events version 5.0 [CTCAE v.5]) and post-procedure pain (visual analog scale [VAS] scale) will be assessed.

TABLE 4. Trial procedures

Procedures	Inclusion	Therapy	Follow-up examinations		
	Day 0	Day 1	Day 3	Day 8	Day 31
Informed consent	X				
Concurrent treatments ¹	X				
Clinical examination	X		X	X	X
Complete blood count, biochemistry, serum cytokines	X ²		X	X	X
Coagulation profile	X ²				
Digital imaging of the tumor and tumor measurement	X ³	X	X	X	X
Immune profile determination ⁴	X		X	X	X
Saliva sample and a skin swab from the location of therapy	X	X	X	X	X
EORTC QLQ-C30	X			X	X
ECOG	X				
Examination prior to anesthesia ⁵	X				
pHIL12 GET		X			
Pain assessment in accordance with the VAS scale		X	X	X	X
CTCAE v.5		X	X	X	X
Punch biopsy				X	X ⁶
Excision of tumor lesion					X ⁷

¹ A detailed description of concurrent treatments (name of the medicinal products, dosage and treatment protocol, beginning of the treatment and reason of the treatment).

² Complete blood count, biochemistry and coagulation profile must be carried out after the inclusion examination, no more than 7 calendar days prior to therapy.

³ Temporary measurement of the size of the tumor and initial imaging must be carried out no more than 7 days prior to therapy.

⁴ Peripheral blood mononuclear cells will be examined with flow cytometry to determine content of different subgroups.

⁵ Prior to therapy, patients will be examined and assessed by anesthesiologists in accordance with the ASA scale.

⁶ Punch biopsy will be performed in case of complete response.

⁷ Tumor lesion will be excised if tumor will NOT completely respond to the treatment.

CTCAE = Common Terminology Criteria for Adverse Events; ECOG = Eastern Cooperative Oncology Group; EORTC QLQ-C30 = European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire C30; GET = gene electrotransfer; VAS scale = visual analog scale

Post-treatment analgesia will be prescribed by the principal investigator in accordance with standard practice. The principal investigator will decide on the need for post-treatment hospitalization regarding the patient's condition following pHIL12 GET therapy and anesthesia in accordance with standard practice following invasive procedures. In this case, hospitalization is not considered as a negative side effect.

Course of the study

All of the trial procedures are listed in Table 4.

Visit 1 (day -6 to 0): Screening and inclusion of the patient into the study

Patients that will meet all inclusion/exclusion criteria, will be acquainted with the course of the study and will sign the informed consent form.

An overview of medical history and treatment (names and dosages of medicinal products, start

date and reason for treatment, and therapy duration) will be done. The investigator will collect the patient's medical history documents with a special emphasis on oncological diseases, previous treatments and response to therapies thus far. A clinical overview will include the measurement of weight, height, blood pressure, pulse. A complete blood count, biochemistry and coagulation profile will be determined at visit 1. Digital imaging of tumor will be performed. The tumor location and its size will be determined. Peripheral blood mononuclear cells will be examined with flow cytometry to determine the content of different subgroups. A saliva sample and skin swabs from the location of the planned application of plasmid DNA will be collected. The quality of life questionnaire (European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire C30, EORTC QLQ-C30) will be completed. EORTC QLQ-C30 is a validated questionnaire for the assessment of quality of life in oncology patients with different cancer types in four areas (physical condition, social/fam-

ily condition, psychological condition, functional condition).²⁵ As part of the questionnaire, patients answer on a four-point scale: not at all/a little/quite a bit/very much or with scores from 1–4. Patient performance status will be evaluated in accordance with Eastern Cooperative Oncology Group (ECOG) criteria. Patients given a score of 0, 1 or 2 are eligible to participate in the clinical study.

In all patients, the eligibility status for anesthesia will be evaluated with the standard pre-anesthetic procedure (ASA scale). Patients not eligible for general anesthesia or sedation, will not be included in the clinical study.

Visit 2 (day 1): Therapy with pHIL12 GET

No more than 7 calendar days may pass from the visit 1 to the visit 2. On visit 2, a follow-up examination will include: confirmation of the eligibility of the patient to continue treatment in the clinical study, the measurement and digital imaging of the tumor, therapy with pHIL12 GET, pain assessment in accordance with Visual Analog Scale (VAS) criteria following treatment, collection of saliva sample and skin swabs from the location of plasmid DNA application, monitoring toxicity and adverse events regarding the CTCAE v.5 criteria.

Visit 3 (day 3): Follow-up examination 2 days after the treatment

On visit 3, a follow-up examination will include: pain assessment in accordance with VAS criteria following treatment, a complete blood count and biochemistry, a blood draw for determining the patient's immune profile (PBMC and subgroups of lymphocytes T), collection of saliva sample and skin swabs from the location of the application of plasmid DNA, monitoring toxicity and adverse events regarding the CTCAE v.5 criteria, the measurement and digital imaging of the tumor.

Visit 4 (day 8 after the treatment): Follow-up examination 7–10 days after the treatment

Visit 4 may vary ± 3 day from the planned visit. On visit 4, a follow-up examination of the patient will include: a clinical examination, pain assessment in accordance with VAS criteria following treatment, a complete blood count and biochemistry, a blood draw for determining the patient's immune profile (PBMC and subgroups of lymphocytes T), collection of saliva sample and skin swabs from the location of the plasmid DNA application, monitoring

toxicity and adverse events regarding the CTCAE v.5 criteria, measurement and digital imaging of the tumor, punch biopsy to determine the efficiency of transfection.

Visit 5 (day 31): Follow-up examination 30 days after treatment

Visit 5 may vary ± 3 day from the planned visit. On visit 5, a follow-up examination of the patient will include: a clinical examination, pain assessment in accordance with VAS criteria following treatment, a complete blood count and biochemistry, a blood draw for determining the patient's immune profile (PBMC and subgroups of lymphocytes T), collection of saliva sample and skin swabs from the location of the plasmid DNA application, monitoring toxicity and adverse events regarding the CTCAE v.5 criteria, the measurement and digital imaging of the tumor, quality of life questionnaires (EORTC QLQ-C30), preliminary evaluation of treatment effectiveness according to the RECIST v1.1 criteria, punch biopsy for assessment of viability of the tumor. Tumors that will not respond to the treatment completely will be excised on day 30 after the treatment. Patients will be instructed to immediately report new symptoms to the doctor.

Follow-up after the therapy

The endpoint of clinical trial is 30 days after the treatment (visit 5). Nevertheless, the patients will be followed up in accordance with national Recommendation for diagnosis, treatment and follow-up of patients with basal cell carcinoma and Guidance on follow up on patients administered with gene therapy medicinal products.^{26,27} The clinical follow up will be performed 3, 6 and 12 months after the treatment. Since the plasmid DNA is associated with low risk of delayed adverse reaction the yearly follow up will be arranged in form of the questionnaire forwarded to the patient (as defined in Guidance on follow up on patients administered with gene therapy medicinal products).

Endpoints evaluation criteria

Safety of intratumoral pHIL12 GET

At each visit, the investigators will note all adverse events (Adverse Event - AE and Serious Adverse Event - SAE) in the appropriate Clinical Report Form (CRF), from the inclusion of the patient into the clinical study until the end of patient follow-up. The investigator will evaluate and record:

- Symptoms or a diagnosis associated with AE;
- The date of the beginning and end of AE;
- The severity of symptoms;
- A causal link to the disease or therapy in a clinical trial;
- A description of measures regarding elimination of AE.

Recording adverse reactions of pHIL12 GET will be in the scope of the CTCAE v.5 criteria (NIH, 2017).

The investigator will evaluate the cause-effect link between the adverse event of pHIL12 GET in accordance with the following criteria:

- Not linked to the study;
- Probably not linked to the study;
- Possibly linked to the study;
- Probably linked to the study;
- Surely linked to the study.

The severity of adverse reactions will be evaluated based on the level:

- Grade 1 – mild;
- Grade 2 – moderate;
- Grade 3 - a difficult complication requiring medical care and treatment;
- Grade 4 - a life-threatening condition requiring immediate medical attention;
- Grade 5 – death.

The investigator will follow-up on patients during the presence of the adverse reactions. According to the investigator assessment of the patient condition, the latter could be withdrawn from the clinical study. The reason for withdrawal of treatment within the clinical study will be recorded on the CRF form for monitoring adverse reactions and on the CRF form upon study completion.

All adverse reactions (AR) that might have a link to AE and the clinical study will be reported to the sponsor. If a serious adverse reaction or a suspicion on it (SAR and SUSAR) is recorded during the study, within 24 hours the principal investigator will in writing inform the study coordinator. The latter will further inform the National Centre for Pharmacovigilance of the Agency for Medicinal Products and Medical Devices of the Republic of Slovenia and the Ethics Committee of the Institute of Oncology Ljubljana. The principal investigator will report to the study coordinator within 24 hours on all changes of the condition regarding a serious adverse reaction. In accordance with the regulation, the sponsor shall also submit regular periodic and annual safety reports. In case of death of a patient, the principal investigator will report on this to the sponsor regardless of whether

the death was associated with progressive disease, therapy or the investigational product or with an unrelated.

Assessment of the tolerability of intratumoral pHIL12 GET

The patients will fill out the quality of life questionnaire prior to treatment and in determined intervals following the treatment (Table 4). We will monitor the hospitalization duration and medicinal products required for pain management during hospitalization and at each control examination.

We will use the following questionnaires:

- Quality of life questionnaires EORTC QLQ-C30;
- Pain assessment in accordance with VAS criteria.

Preliminary evaluation of treatment effectiveness according to the RECIST v1.1 Criteria

Preliminary objective tumor response will be assessed in accordance with RECIST v1.1. criteria. The responses to be measured are:

- Complete Response (CR): Disappearance of target lesion;
- Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesion, taking as reference the baseline sum diameters;
- Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesion, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (the appearance of one or more new lesions is also considered progression);
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Pharmacokinetics, pharmacodynamics of the treatment

Levels of plasmid pHIL12 and IL-12 and IFN- γ protein levels will be evaluated in tumor biopsies. Serum levels of IL-12 cytokine will be monitored during each visit. As a part of the clinical study, the swabs will be taken prior to treatment, after treatment and at control checkups from the location of

plasmid DNA application. Furthermore, the saliva samples will be collected at each visit. Saliva samples and skin swabs will be used to determine the shedding of plasmid DNA. From the samples, the presence of plasmid DNA will be evaluated with the quantitative PCR method in real time. Changes in blood parameters will be followed at each visit determining complete blood count, biochemistry and immune subgroups profile.

Sample size and statistical analysis

The study is exploratory; therefore, no formal sample size calculation was performed. The design (3 + 3 design) and the corresponding sample size are usual for Phase I trials in oncology. All statistical analyses will be descriptive.

Discussion

The current trial is designed to evaluate the safety and tolerability of the intratumoral pHIL12 plasmid DNA gene electrotransfer. The drug dosage that will be established as safe and capable to elicits local immune response will be used for design of next clinical trials.

The gene electrotransfer utilizing IL-12 plasmid DNA was tested extensively in preclinical models and was also used in clinical trials before. To our knowledge, one Phase 1 clinical study (NCT00323206)⁴ and four Phase 2 clinical studies have been conducted in USA in patients with malignant melanoma, cutaneous lymphoma, squamous cell carcinoma of the head and neck, and Merkel cell cancer (NCT01502293, NCT01579318, NCT02345330, NCT01440816).^{9,28} Further, four new studies (NCT03132675, NCT04526730, NCT03567720, NCT03823131) are active, also in mucosal head and neck squamous cell carcinoma patients, to evaluate a combination of GET of plasmid DNA encoding IL-12 in combination with pembrolizumab or nivolumab.

The above-mentioned Phase 2 clinical studies were performed with tavokinogene telseplasmid (TAVO™), a plasmid encoding IL-12 produced by OncoSec Medical Incorporated (USA). It is a plasmid that encodes genes for the p35 and p40 subunits of the heterodimeric human IL-12 protein separated by an internal ribosome entry site. Constitutive expression of the subunits is driven by a single cytomegalovirus promoter. Once the plasmid is introduced into a mammalian cell, a functional IL-12 p70 protein is expressed and secreted

into the tumor microenvironment. Intratumoral GET of accessible lesions is performed using an *in situ* EP device.⁸ The TAVO™ plasmid contains a kanamycin resistance gene as the selection gene for the production process, a feature that is discouraged by EMA.

Plasmid pHIL12, like TAVO™, is a plasmid DNA, encoding for p35 and p40 subunits of the heterodimeric human IL-12 protein. However, the plasmid backbone of pHIL12 is different, since we aimed for the plasmid devoid of antibiotic resistance marker and for the treatment-inducible, tumor-specific promoter, whereas the transgene product is the same in both plasmids, a functional IL-12 p70 protein. In pHIL12 plasmid, operator-repressor titration ORT® technology, which is based on providing the titration of repressor of essential gene for propagation of bacteria in plasmid, was used. Multiple operators present on the plasmid titrate the repressor leading to expression of essential gene. In our case, the essential gene in bacteria was *dapD*, which was under transcriptional control of lac operator/promotor (lacO/P). Genome encoded *LacI* repressor prevents bacterial growth, unless transformed with plasmid containing the lac operator (lacO) that titrates the LacI repressor from the operator.²⁹ In addition, since the use of pHIL12 GET is foreseen as an adjuvant treatment to local ablative therapies, to increase their local and elicit a systemic response, the IL12 coding sequence was placed under the transcriptional control of an inducible p21 (or cyclin dependent kinase inhibitor 1A (CDKN1A)) promoter that can be activated by the hypoxic tumor microenvironment and/or by the genotoxic stress induced by local ablative therapies, such as tumor irradiation of electrochemotherapy.¹⁶

Based on preclinical data for TAVO™ that are adequately comparable to pHIL12 and since the expression product and a mode of action are the same (i.e. protein IL-12), we decided to use the same doses as in the above clinical studies as a basis for selecting a starting dose in non-clinical study and in this first-in-human phase I study.

The treatment protocol with TAVO™ was designed as a repetitive protocol at days 1, 5 and 8, with possible repetitive cycles at 6- or 12-weeks intervals. However, our intent is to use pHIL12 as adjuvant immunotherapeutic to the established local ablative therapies. As already reported, ablation of tumors induces local immune response with immunogenic cell death.^{30,31} Therefore, studies combining local ablative therapies like radiotherapy or electrochemotherapy can be boosted from local to

locoregional or even systemic response by immune checkpoint inhibitors. The recent study combining electrochemotherapy with pembrolizumab, demonstrated that this approach is feasible. The patients treated with pembrolizumab and ECT experienced lower disease progression rates and longer survival than those who received pembrolizumab alone.³² Similar approach could be by immunostimulation using pHIL12 intratumoral GET. Further, different studies combining tumor irradiation and immune checkpoint inhibitors demonstrated the same.^{33–36} Therefore, in the future clinical trials pHIL12 is envisioned as an immunostimulating component to *in situ* vaccinating effect of local ablative therapies such as electrochemotherapy or radiotherapy. We believe that in the frame of such treatment combination, a single treatment with IL-12 GET would be sufficient to elicit pronounced antitumor effect.

Conclusions

The designed first-in-human clinical trial is aimed at evaluation of safety and tolerability of pHIL12 GET, thus setting the stage for the use of pHIL12 GET as an adjuvant treatment to local ablative therapies, to increase their local and elicit a systemic response.

Author contributions

GS, AG, MC, PS, MB, TJ and BM contributed to conception and design of the study. MB, TJ and GS wrote the first draft of the manuscript. AG, MC, PS and BM wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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